

8-2015

MICROCOSM EVALUATION OF ENHANCED BIOREMEDIATION FOR AN INDUSTRIAL SITE CONTAMINATED WITH CHLORINATED ETHENES, CHLORINATED BENZENES, AND BENZENE

Christopher Moss

Clemson University, cmoss2@g.clemson.edu

Follow this and additional works at: https://tigerprints.clemson.edu/all_theses

 Part of the [Engineering Commons](#)

Recommended Citation

Moss, Christopher, "MICROCOSM EVALUATION OF ENHANCED BIOREMEDIATION FOR AN INDUSTRIAL SITE CONTAMINATED WITH CHLORINATED ETHENES, CHLORINATED BENZENES, AND BENZENE" (2015). *All Theses*. 2231.

https://tigerprints.clemson.edu/all_theses/2231

This Thesis is brought to you for free and open access by the Theses at TigerPrints. It has been accepted for inclusion in All Theses by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.

**MICROCOSM EVALUATION OF ENHANCED BIOREMEDIATION
FOR AN INDUSTRIAL SITE CONTAMINATED WITH
CHLORINATED ETHENES, CHLORINATED BENZENES,
AND BENZENE**

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Environmental Engineering and Science

by
Christopher Moss
August 2015

Accepted by:
Dr. David L. Freedman, Committee Chair
Dr. Elizabeth R. Carraway
Dr. Kevin T. Finneran

ABSTRACT

Groundwater contaminants at an industrial site in South Carolina include tetrachloroethene (PCE), trichloroethene (TCE), *cis*-1,2-dichloroethene (cDCE), vinyl chloride (VC), 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2-dichlorobenzene (1,2-DCB), 1,4-dichlorobenzene (1,4-DCB), chlorobenzene (CB), and benzene. The source areas at the site include a tank farm area, a grease trap area, the former wastewater lagoons (WWTP) and the former oil retention pond (ORP). A microcosm evaluation was performed to determine the potential for bioremediation to treat the organic contaminants in the four source areas (within the saturated zone) and in a downgradient portion of the groundwater plume. The objectives were to evaluate 1) anaerobic treatment at the source zones using biostimulation with lactate or emulsified vegetable oil; 2) bioaugmentation with a commercial chloroethene respiring culture; and 3) addition of zero valent iron (ZVI) with lactate. Additional microcosms were prepared using the same conditions and initially incubated anaerobically; these were then converted to aerobic conditions after the chlorinated ethenes were consumed. Controls included anaerobic conditions without amendments, aerobic conditions without amendments, water controls, and autoclaved controls. For the downgradient groundwater microcosms, the same treatments were used except for the two sets with ZVI. Triplicate bottles were prepared for each treatment, resulting in a total of 147 microcosms.

Based on observations made during approximately 400 days of monitoring, the following conclusions were reached:

- Sequential anaerobic/aerobic bioremediation is a viable treatment approach for

the WWTP, ORP, and downgradient locations. PCE and TCE can be efficiently dechlorinated to ethene under anaerobic conditions and the aromatic compounds can be oxidized under subsequent aerobic conditions.

- It is feasible to use only anaerobic biostimulation to remove PCE and TCE for the WWTP and ORP locations, whereas bioaugmentation will be required for the downgradient location. Consideration should also be given to using bioaugmentation for the WWTP and ORP locations, as this will accelerate removal of PCE and TCE so that a switch to aerobic conditions can occur more quickly.
- There was no compelling evidence in support of anaerobic biodegradation of the aromatic compounds, either via reductive dechlorination to benzene or via anaerobic oxidation. However, aerobic biodegradation of the aromatic compounds is feasible for the WWTP, ORP, and downgradient locations.
- Development of an aerobic enrichment culture for biodegradation of the aromatic contaminants at the WWTP, ORP and downgradient locations is advisable. Aerobic aromatic degraders are present at the WWTP. Groundwater from this location can be used to develop an indigenous bioaugmentation culture. The process of enrichment can either be done in an on-site reactor or by an off-site vendor. Bioaugmentation with an aerobic enrichment culture will significantly improve the rate of aerobic treatment of the aromatic compounds at the WWTP, ORP and downgradient locations.
- Without additional evaluation, bioremediation alone is not feasible for the tank

farm and grease trap locations. ZVI shows promise for treating the PCE and TCE, although additional study is warranted to find an appropriate dose. Also, additional studies are needed to ascertain if aerobic biological treatment would be feasible to address the aromatic compounds following ZVI treatment of PCE and TCE.

- 1,2,4-TCB is at least partially responsible for the lack of PCE and TCE reductive dechlorination in the grease trap microcosms. This conclusion is based on the inhibition test, which showed that the presence of high concentrations of 1,2,4-TCB inhibited KB-1. Further experiments should be conducted to determine the effectiveness of aerobic removal of aromatics, specifically 1,2,4-TCB, followed by anaerobic treatment.

DEDICATION

This thesis is dedicated to my family my mother Rose, my father Dennis, my brother Tim and his wife Becky for their unending support in all my endeavors. Also to the friends I have found in Clemson and the experiences they've shared with me.

ACKNOWLEDGEMENTS

I want to thank Dr. Freedman for giving me the opportunity to perform this research. I appreciate Dr. Finneran and Carraway for being members of my committee. I'd also like to acknowledge Rong Yu for completion of the qPCR analysis and Jin Gao for assistance in the lab and preparation of the microcosms. As well as TRC Environmental for funding the research.

TABLE OF CONTENTS

	Page
TITLE PAGE.....	i
ABSTRACT.....	ii
DEDICATION.....	v
ACKNOWLEDGEMENTS	vi
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xxvii
1.0 INTRODUCTION AND OBJECTIVES.....	1
1.1 Chlorinated Ethenes.....	1
1.2 Chlorinated Benzenes and Benzene	4
1.3 Objectives	7
2.0 MATERIALS AND METHODS	9
2.1 Sample Locations	9
2.2 Chemicals and Medium	9
2.3 Experimental Design	10
2.4 Microcosm Preparation and Operation	11
2.4.1 Preparation	11
2.4.2 Operation	15
2.5 Analytical Procedures	16
3.0 RESULTS	20
3.1 Wastewater Treatment Plant	21
3.1.1 Autoclaved Controls	21
3.1.2 Anaerobic Only Treatments	21
3.1.3 Aerobic Only Treatment	23
3.1.4 Anaerobic Followed by Aerobic Treatments	24
3.2 Oil Retention Pond.....	25
3.2.1 Autoclaved Controls	25
3.2.2 Anaerobic Only Treatments	26
3.2.3 Aerobic Only Treatment	27
3.2.4 Anaerobic Followed by Aerobic Treatments	28
3.3 Tank Farm	30
3.3.1 Autoclaved Controls	30

TABLE OF CONTENTS (CONTINUED)

	Page
3.3.2 Anaerobic Only Treatments	30
3.3.3 Aerobic Only Treatment	31
3.3.4 Anaerobic Followed by Aerobic Treatments	32
3.4 Grease Trap	33
3.4.1 Autoclaved Controls	33
3.4.2 Anaerobic Only Treatments	33
3.4.3 Aerobic Only Treatment	35
3.4.4 Anaerobic Followed by Aerobic Treatments	35
3.4.5 Soil Analysis	36
3.5 Downgradient	36
3.5.1 Autoclaved Controls	36
3.5.2 Anaerobic Only Treatments	37
3.5.3 Aerobic Only Treatment	38
3.5.4 Anaerobic Followed by Aerobic Treatments	39
3.6 qPCR Estimation of <i>Dehalococcoides</i> and Halorespiratory Genes	40
3.7 Aerobic Enrichment Culture	41
3.8 Inhibition	42
3.9 Summary	44
3.9.1 Wastewater Treatment Plant	44
3.9.2 Oil Retention Pond	47
3.9.3 Tank Farm and Grease Trap	48
3.9.4 Downgradient	52
 4.0 DISCUSSION	 53
4.1 Effect of Hydrology on Bioremediation	53
4.2 Remediation Techniques	54
4.3 Amendments for Remediation	55
 5.0 CONCLUSIONS AND RECOMMENDATIONS	 57
5.1 Conclusions	57
5.2 Recommendations	58
 TABLES	 60
 FIGURES	 73
 APPENDICES	 132
Appendix A: Media Preparation	133
Anaerobic MSM	133
Aerobic MSM	136
Appendix B: Response Factors	137

TABLE OF CONTENTS (CONTINUED)

	Page
Appendix C: Low Concentration Aromatic Graphs	144
REFERENCES.....	172

LIST OF TABLES

Table 2.1. . Sources and purity of most chemicals used	61
Table 2.2. Components of the anaerobic MSM used in the inhibition test	62
Table 2.3. Experimental design.....	63
Table 2.4. Targeted initial concentrations of contaminants based on historical monitoring data.	64
Table 2.5. Volumes withdrawn and added to achieve targeted contaminant concentrations.	65
Table 2.6. Factors to convert mass per bottle to aqueous phase concentrations.	66
Table 2.7. Components of qPCR reaction mix.....	67
Table 3.1. Inorganic constituents in Grease Trap soil.	68
Table 3.2. qPCR results for groundwater and lactate-amended anaerobic microcosms.	69
Table 3.3. Summary of microcosm performance for each location and each treatment, with respect to the chlorinated ethenes (CE) and aromatic compounds (ARO).	70
Table3.4. Sulfate concentrations in microcosms, including a comparison between electron donor required for reduction and electron donor added in each treatment	71
Table 5.1 Ratio of lactate added as donor to demand for PCE and TCE reduction to ethene.	72
Table B.1. Oxygen response factor	137

LIST OF FIGURES

Figure 3.1. a) Water controls for 1,2,4-TCB; b) 1,2-DCB, 1,4-DCB, CB and benzene; and c) PCE and TCE. Error bars represent standard deviation.	74
Figure 3.2. Results for the triplicate WTTP autoclaved control microcosms; error bars represent standard deviations; a) aromatic compounds at high concentrations; and b) chlorinated ethenes.....	75
Figure 3.3. Results for the triplicate WTTP unamended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene and methane.....	76
Figure 3.4. Results for the triplicate WTTP anaerobic lactate-amended microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate.	77
Figure 3.5. Results for the triplicate WTTP EVO-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of EVO.	78
Figure 3.6. Results for the triplicate WTTP bioaugmented anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate and stars indicate addition of the KB-1 bioaugmentation culture.....	79
Figure 3.7. Results for the triplicate WTTP ZVI and lactate-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate.	80
Figure 3.8. Results for the triplicate WTTP unamended aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Tiger paws indicate re-addition of chlorinated benzenes and benzene.....	81

LIST OF FIGURES (CONTINUED)

	Page
Figure 3.9. Results for the triplicate WTTP lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate; stars indicate addition soil from WW-AER microcosms; and the tiger paws indicate re-addition of chlorinated benzenes and benzene.....	82
Figure 3.10. Results for the triplicate WTTP bioaugmented anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture and yellow stars indicate addition of soil from the WW-AER microcosms.	83
Figure 3.11. Results for the WTTP ZVI and lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate; stars indicate addition of soil from WW-AER microcosm #2, and tiger paws indicate re-addition of chlorinated benzenes and benzene.	84
Figure 3.12. Results for the triplicate ORP autoclaved control microcosms; error bars represent standard deviations; a) CB and benzene; and b) PCE and TCE.....	85
Figure 3.13. Results for the triplicate ORP unamended anaerobic microcosms; a) CB and benzene; and b) PCE, TCE and methane.	86
Figure 3.14. Results for the ORP anaerobic lactate-amended microcosm #1; a) CB and benzene; and b) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate.	87
Figure 3.14 (continued). Results for the ORP anaerobic lactate-amended microcosm #2; c) CB and benzene; and d) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate.	88

Page

LIST OF FIGURES (CONTINUED)

	Page
Figure 3.14 (continued). Results for the ORP anaerobic lactate-amended microcosm #3; e) CB and benzene; and f) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate.	89
Figure 3.15. Results for the triplicate ORP EVO-amended anaerobic microcosms; a) CB and benzene; and b) chlorinated ethenes and methane. Arrows indicate addition of EVO.	90
Figure 3.16. Results for the triplicate ORP bioaugmented anaerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate and stars indicate addition of the KB-1 bioaugmentation culture.	91
Figure 3.17. Results for the triplicate ORP ZVI and lactate-amended anaerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate.	92
Figure 3.18. Results for the triplicate ORP unamended aerobic microcosms; a) CB and benzene; and b) PCE, TCE, and methane. Yellow stars indicate addition of soil and groundwater from the WW-AER microcosms.	93
Figure 3.19. Results for the triplicate ORP lactate-amended anaerobic/aerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate.	94
Figure 3.20. Results for the triplicate ORP bioaugmented anaerobic/aerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture; yellow stars indicate addition of soil and groundwater from the WW-AER microcosms; and tiger paws indicate re-addition of chlorinated benzenes and benzene.	95

LIST OF FIGURES (CONTINUED)

	Page
Figure 3.21. Results for the ORP ZVI and lactate-amended anaerobic/aerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate an addition of lactate, stars indicate addition of soil from WW-AER series and tiger paws indicate addition of chlorinated benzenes and benzene.	96
Figure 3.22. Results for the triplicate Tank Farm autoclaved control microcosms; error bars represent standard deviations; a) aromatic compounds at high concentrations; and b) PCE and TCE.	97
Figure 3.23. Results for the triplicate Tank Farm unamended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE.	98
Figure 3.24. Results for the triplicate Tank Farm anaerobic lactate-amended microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE. Arrows indicate addition of lactate.	99
Figure 3.25. Results for the triplicate Tank Farm EVO-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Arrows indicate addition of EVO.	100
Figure 3.26. Results for the triplicate Tank Farm bioaugmented anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Black arrows indicate addition of lactate, green arrows indicate addition of corn syrup, and stars indicate addition of the KB-1 bioaugmentation culture.	101
Figure 3.27. Results for the triplicate Tank Farm ZVI and lactate-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and ethane. Arrows indicate addition of lactate.	102

LIST OF FIGURES (CONTINUED)

	Page
Figure 3.28. Results for the triplicate Tank Farm unamended aerobic microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE. Stars indicate addition of 2.0 mL from the WWTP AER microcosm #2.	103
Figure 3.29. Results for the triplicate Tank Farm lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.	104
Figure 3.30. Results for the triplicate Tank Farm bioaugmented anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE. Black arrows indicate addition of lactate, green arrows indicate addition of corn syrup and stars indicate addition of KB-1 culture. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.	105
Figure 3.31. Results for the Tank Farm ZVI and lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and ethane. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.	106
Figure 3.32. Results for the triplicate Grease Trap autoclaved control microcosms; error bars represent standard deviations; a) aromatic compounds at high concentrations; and b) PCE and TCE.....	107
Figure 3.33. Results for the triplicate Grease Trap unamended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes.	108
Figure 3.34. Results for the triplicate Grease Trap anaerobic lactate-amended microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Arrows indicate addition of lactate.	109

LIST OF FIGURES (CONTINUED)

	Page
Figure 3.35. Results for the triplicate Grease Trap EVO-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Arrows indicate addition of EVO.	110
Figure 3.36. Results for the triplicate Grease Trap bioaugmented anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Black arrows indicate addition of lactate, green arrows indicate addition of corn syrup and stars indicate addition of KB-1 culture.	111
Figure 3.37. Results for the triplicate Grease Trap ZVI and lactate-amended anaerobic microcosms; a) chlorinated benzenes at high concentrations; and b) chlorinated ethenes, ethene, and ethane. Arrows indicate addition of lactate.	112
Figure 3.38. Results for the triplicate Grease Trap unamended aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Stars indicate addition of 2.0 mL from the WWTP AER microcosm #2.	113
Figure 3.39. Results for the triplicate Grease Trap lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.....	114
Figure 3.40. Results for the triplicate Grease Trap bioaugmented anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Black arrows indicate addition of lactate, green arrows indicate addition of corn syrup and stars indicate addition of KB-1 culture. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.....	115
Figure 3.41. Results for the Grease Trap ZVI and lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high	

LIST OF FIGURES (CONTINUED)

	Page
concentrations; and b) chlorinated ethenes, ethene, and ethane. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.	116
Figure 3.42. Results for the triplicate downgradient plume autoclaved control microcosms; error bars represent standard deviations; a) aromatic compounds; and b) chlorinated ethenes.	117
Figure 3.43. Results for the triplicate downgradient plume unamended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene and methane.	118
Figure 3.44. Results for the triplicate downgradient plume anaerobic lactate-amended microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate.	119
Figure 3.45. Results for the triplicate downgradient plume EVO-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of EVO.	120
Figure 3.46. Results for the triplicate downgradient plume bioaugmented anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate and stars indicate addition of the KB-1 bioaugmentation culture.	121
Figure 3.47. Results for downgradient plume unamended aerobic microcosm #1; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethane, and methane. Tiger paws indicate addition of aromatic compounds.	122
Figure 3.47 (continued). Results for downgradient plume unamended aerobic microcosm #2; c) aromatic compounds at high concentrations; and d) chlorinated ethenes, ethane, and methane. Tiger paws indicate addition of aromatic compounds.	123

LIST OF FIGURES (CONTINUED)

	Page
Figure 3.47 (continued). Results for downgradient plume unamended aerobic microcosm #3; e) aromatic compounds at high concentrations; and f) chlorinated ethenes, ethane, and methane. Tiger paws indicate addition of aromatic compounds.	124
Figure 3.48. Results for the triplicate downgradient plume lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.	125
Figure 3.49. Results for downgradient plume bioaugmented anaerobic/aerobic microcosm #1; a) aromatic compounds at high concentrations; and b) ethenes, ethane, and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture and yellow stars indicate addition of soil and groundwater from the WW-AER microcosms.	126
Figure 3.49 (continued). Results for downgradient plume bioaugmented anaerobic/aerobic microcosm #2; c) aromatic compounds at high concentrations; and d) ethenes, ethane, and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture, yellow stars indicate addition of soil and groundwater from the WW-AER microcosms, and tiger paws indicate re-addition of aromatic compounds.	127
Figure 3.49 (continued). Results for downgradient plume bioaugmented anaerobic/aerobic microcosm #2; e) aromatic compounds at high concentrations; and f) ethenes, ethane, and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture, yellow stars indicate addition of soil and groundwater from the WW-AER microcosms, and tiger paws indicate re-addition of aromatic compounds.	128
Figure 3.50. Results for the aerobic enrichment culture. Average of triplicate bottles. Stars indicate addition of 1 mL soil from WWTP aerobic microcosms.	129

LIST OF FIGURES (CONTINUED)

	Page
Figure 3.51. Results for the inhibition test containing chlorinated ethenes only. Averages of triplicate bottles .	129
Figure 3.52. Results for the inhibition test containing aromatics at tank farm levels. Graphs show averages of triplicates. a) concentration of aromatics present, b) concentrations of chlorinated ethenes, ethene and methane. Arrows indicate an addition of lactate	130
Figure 3.53. Results for the inhibition test containing aromatics at grease trap levels. Graphs show averages of triplicates. a) concentration of aromatics present, b) concentrations of chlorinated ethenes, ethene and methane. Arrows indicate an addition of lactate.	131
Figure B.1. Calibration curve for methane for the HP 5890 Series II GC.	138
Figure B.2. Calibration curve for ethane for the HP 5890 Series II GC.	138
Figure B.3. Calibration curve for ethene for the HP 5890 Series II GC.	139
Figure B.4. Calibration curve for vinyl chloride for the HP 5890 Series II GC.	139
Figure B.5. Calibration curve for cis-dichloroethene for the HP 5890 Series II GC.	140
Figure B.6. Calibration curve for trichloroethene for the HP 5890 Series II GC.	140
Figure B.7. Calibration curve for tetrachloroethene for the HP 5890 Series II GC.	141
Figure B.8. Calibration curve for benzene for the HP 6890 GC.	141

LIST OF FIGURES (CONTINUED)

	Page
Figure B.9. Calibration curve for chlorobenzene for the HP 6890 GC.....	142
Figure B.10. Calibration curve for 1,2 dichlorobenzene for the HP 6890 GC.....	142
Figure B.11. Calibration curve for 1,3 dichlorobenzene for the HP 6890 GC.....	143
Figure B.12. Calibration curve for 1,2,4-trichlorobenzene for the HP 6890 GC.....	143
Figure C.1. Results for the triplicate WTTP autoclaved control microcosms; low concentration chlorinated benzenes. Error bars represent standard deviations.....	144
Figure C.2. Results for the triplicate WTTP unamended anaerobic microcosms; low concentration chlorinated benzenes.....	144
Figure C.3. Results for the triplicate WTTP anaerobic lactate-amended microcosms; low concentration chlorinated benzenes. Arrows indicate additon of lactate.	145
Figure C.4. Results for the triplicate WTTP EVO-amended anaerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of EVO.	145
Figure C.5. Results for the triplicate WTTP bioaugmented anaerobic microcosms. low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of KB-1 bioaugmentation culture.....	146
Figure C.6. Results for the triplicate WTTP ZVI and lactate amended anaerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.	146
Figure C.7. Results for the triplicate WTTP unamended aerobic microcosms; low concentration chlorinated benzenes. Tiger paws indicate re-addition of chlorinated benzenes.	147

LIST OF FIGURES (CONTINUED)

	Page
Figure C.8. Results for the triplicate WTTP lactate-amended anaerobic/aerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of soil from WW-AER microcosms. Tiger paws indicate re-addition of chlorinated benzenes.	147
Figure C.9. Results for the triplicate WTTP bioaugmented anaerobic/aerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Blue stars indicate addition of KB-1 culture and yellow stars indicate addition of soil from the WW-AER microcosms. A Tiger paw indicates re-addition of chlorinated benzenes.....	148
Figure C.10. Results for the triplicate WTTP ZVI and lactate-amended anaerobic/aerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of soil from WW-AER microcosms. Tiger paws indicate re-addition of chlorinated benzenes.	148
Figure C.11. Results for the triplicate ORP autoclaved microcosms: averages of triplicate bottles, Low concentrations chlorinated benzenes. Error bars represent standard deviations.	149
Figure C.12. Results for the triplicate ORP anaerobic unamended microcosms: averages of triplicate bottles, Low concentrations chlorinated benzenes.	149
Figure C.13. Results for the ORP anaerobic lactate amended a) microcosm #1, low concentration chlorinated benzenes. b) Microcosm #2, low concentration chlorinated benzenes. Arrows indicate addition of lactate.	150
Figure C.13 (continued). Results for the ORP anaerobic lactate amended c) microcosm #3, low concentration chlorinated benzenes. Arrows indicate addition of lactate.	151

LIST OF FIGURES (CONTINUED)

	Page
Figure C.14. Results for the triplicate ORP anaerobic EVO amended, low concentration chlorinated benzenes. Arrows indicate addition of EVO.	151
Figure C.15. Results for the triplicate ORP anaerobic bioaugmented, low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of KB-1 culture.	152
Figure C.16. Results for the triplicate ORP anaerobic ZVI and lactate amended microcosms, low concentration chlorinated benzenes. Arrows indicate addition of lactate.	152
Figure C.17. Results for the ORP aerobic unamended a) microcosm #1, b) microcosm #2 low concentration chlorinated benzenes. Stars indicate addition of soil from WW-AER.	153
Figure C.17 (continued). Results for the ORP aerobic unamended c) microcosm #3, low concentration chlorinated benzenes. Stars indicate addition of soil from WW-AER.	154
Figure C.18. Results for the triplicate ORP anaerobic/aerobic lactate amended microcosms, low concentration chlorinated benzenes. Arrows indicate addition of lactate.	154
Figure C.19. Results for the triplicate ORP anaerobic/aerobic bioaugmented amended microcosms, low concentration chlorinated benzenes. Arrows indicate addition of lactate. Blue stars indicate addition of KB-1 culture. Yellow stars indicate addition of soil from WW-AER. Tiger paws indicate re-addition of chlorinated benzenes.	155
Figure C.20. Results for the triplicate ORP anaerobic/aerobic ZVI and lactate amended microcosms, low concentration chlorinated benzenes. Arrows indicate addition of lactate. Yellow stars indicate addition of soil from WW-AER. Tiger paws indicate re-addition of chlorinated benzenes.	155

LIST OF FIGURES (CONTINUED)

	Page
Figure C.21. Results for the triplicate tank farm autoclaved control microcosms; low concentration chlorinated benzenes. Error bars represent standard deviations.	156
Figure C.22. Results for the triplicate tank farm anaerobic unamended microcosms; low concentration chlorinated benzenes.	156
Figure C.23. Results for the triplicate tank farm anaerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.	157
Figure C.24. Results for the triplicate tank farm anaerobic EVO amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of EVO.	157
Figure C.25. Results for the triplicate tank farm anaerobic bioaugmented microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate. Green arrows indicate addition of corn syrup. Stars indicate addition of KB-1 culture.	158
Figure C.26. Results for the triplicate tank farm anaerobic ZVI and lactate amended microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate.	158
Figure C.27. Results for the triplicate tank farm aerobic unamended microcosms; low concentration chlorinated benzenes. Stars indicate addition of soil from ww-aer.	159
Figure C.28. Results for the triplicate tank farm anaerobic/aerobic lactate amended microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate.	159
Figure C.29. Results for the triplicate tank farm anaerobic/aerobic bioaugmented microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate. Green arrows indicate addition of corn syrup. Stars indicate addition of KB-1 culture.	160

LIST OF FIGURES (CONTINUED)

	Page
Figure C.30. Results for the triplicate tank farm anaerobic ZVI and lactate amended microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate.	160
Figure C.31. Results for the triplicate grease trap autoclaved control microcosms; low concentration chlorinated benzenes. Error bars represent standard deviations.	161
Figure C.32. Results for the triplicate grease trap anaerobic unamended microcosms; low concentration chlorinated benzenes.	161
Figure C.33. Results for the triplicate grease trap anaerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.	162
Figure C.34. Results for the triplicate grease trap anaerobic EVO amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of EVO.	162
Figure C.35. Results for the triplicate grease trap anaerobic bioaugmented microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate. Green arrows indicate corn syrup. Stars indicate addition of KB-1 culture.	163
Figure C.36. Results for the triplicate grease trap anaerobic ZVI and lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.	163
Figure C.37. Results for the triplicate grease trap aerobic unamended microcosms; low concentration chlorinated benzenes. Stars indicate addition of soil from ww-aer.	164
Figure C.38. Results for the triplicate grease trap anaerobic/aerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.	164
Figure C.39. Results for the triplicate grease trap anaerobic/aerobic bioaugmented microcosms; low concentration	

LIST OF FIGURES (CONTINUED)

	Page
chlorinated benzenes. Black arrows indicate addition of lactate. Green arrows indicate corn syrup. Stars indicate addition of KB-1 culture.	165
Figure C.40. Results for the triplicate grease trap anaerobic/aerobic ZVI and lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.	165
Figure C.41. Results for the triplicate downgradient plume autoclaved control microcosms; low concentration chlorinated benzenes. Error bars represent standard deviations.	166
Figure C.42. Results for the triplicate downgradient plume anaerobic unamended microcosms; low concentration chlorinated benzenes.	166
Figure C.43. Results for the triplicate downgradient plume anaerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.	167
Figure C.44. Results for the triplicate downgradient plume anaerobic EVO amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of EVO.	167
Figure C.45. Results for the triplicate downgradient plume anaerobic bioaugmented microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of KB-1 culture.	168
Figure C.46. Results for the downgradient plume aerobic unamended a) microcosm #1; low concentration chlorinated benzenes. Tiger paw indicate re-addition of chlorinated benzenes.	168
Figure C.46 (continued). Results for the downgradient plume aerobic unamended b) microcosm #2 and c) microcosm #3; low concentration chlorinated benzenes. Tiger paw indicate re-addition of chlorinated benzenes.	169

LIST OF FIGURES (CONTINUED)

	Page
Figure C.47. Results for the triplicate downgradient plume anaerobic/aerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.	170
Figure C.48. Results for the downgradient plume anaerobic/aerobic bioaugmented a) microcosm #1; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Yellow stars indicate addition of soil from ww-aer. Blue stars indicate addition of KB-1 culture. Tiger paw indicate re-addition of chlorinated benzenes.	170
Figure C.48 (continued). Results for the downgradient plume anaerobic/aerobic bioaugmented microcosm #2 b) and microcosm #3 c); low concentration chlorinated benzenes. Arrows indicate addition of lactate. Yellow stars indicate addition of soil from ww-aer. Blue stars indicate addition of KB-1 culture. Tiger paw indicate re-addition of chlorinated benzenes.	171

LIST OF ABBREVIATIONS

1,2-DCB	1,2-dichlorobenzene
1,2,4-TCB	1,2,4-trichlorobenzene
1,3-DCB	1,3-dichlorobenzene
1,4-DCB	1,4-dichlorobenzene
ABC®	Anaerobic BioChem
CB	chlorobenzene
cDCE	cis-1,2-dichloroethene
EVO	emulsified vegetable oil
GC	gas chromatography
MSM	mineral salts medium
ORP	Oil Retention Pond
PCE	tetrachloroethene
TCE	trichloroethene
VC	vinyl chloride
WWTP	waste water treatment plant
ZVI	zero valent iron

CHAPTER ONE

1.0 INTRODUCTION AND OBJECTIVES

The complexity of hazardous waste site remediation tends to increase as the types of contaminants increases. For example, the groundwater at an industrial waste site in South Carolina has been impacted by a mixture of volatile and semi-volatile organic compounds, including chlorinated ethenes, chlorinated benzenes, and benzene. Tetrachloroethene (PCE), trichloroethene (TCE), *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC) are the main chlorinated ethenes present; 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2-dichlorobenzene (1,2-DCB), 1,4-dichlorobenzene (1,4-DCB) and chlorobenzene (CB) are the main chlorinated benzenes. There are several source areas identified at the site, including a tank farm area (a chemical storage area of the active industrial facility), a grease trap area, former lagoons at the wastewater treatment plant (WWTP), and a former oil retention pond (ORP). The overall objective of this thesis research was to evaluate the potential for using bioremediation to treat these source area organics (within the saturated zone) and a more dilute downgradient groundwater plume.

In order to provide a foundation for the research objectives and experimental plan, a review of the literature on bioremediation of chlorinated ethenes is provided below, followed by chlorinated benzenes and benzene.

1.1 Chlorinated Ethenes

Field monitoring data from the industrial site indicate that reductive dechlorination of chlorinated ethenes is occurring, although the rate of complete

reduction to ethene is not sufficient to prevent migration of the contaminated groundwater offsite. In situations such as these, biostimulation and/or bioaugmentation (i.e., addition of a culture that is capable of using PCE and its daughter products as terminal electron acceptors) may be required. The types of microbes that carry out anaerobic halorespiration of chlorinated ethenes to ethene belong to the *Dehalococcoides* genus. If the aquifer does not have an adequate indigenous population of halorespiring *Dehalococcoides* type microbes, it will be necessary to add them, using a commercially available enrichment culture (Cashwell et al. 2004; Ellis et al. 2000; Lendvay et al. 2003). One of the objectives of this study was to ascertain the likely response to *in situ* biostimulation and bioaugmentation.

Under aerobic conditions, PCE is widely considered to be refractory. TCE will not undergo any appreciable aerobic biodegradation unless primary substrates are present whose metabolism induces oxygenases capable of cometabolically oxidizing TCE. Benzene can serve the role of a primary substrate, since aerobic biodegradation induces the activity of oxygenases. Elango et al. (2011) demonstrated with several pure cultures that CB, 1,2-DCB, and 1,3-DCB also serve as primary substrates for aerobic cometabolism of TCE and cDCE. Indirect evidence from a related microcosm study suggests that aerobic biodegradation of 1,4-DCB and 1,2,4-TCB supports cometabolism of TCE and cDCE (Elango et al. 2010). Microbes and enrichment cultures have been identified that are capable of aerobically biodegrading cDCE as a sole carbon and energy source (Coleman et al. 2002; Giddings et al. 2010; Tiehm et al. 2008), although their distribution in the environment appears to be limited to a few locations. In contrast,

microbes that are able to aerobically biodegrade VC as a sole carbon and energy source are quite common (Coleman et al. 2002; Hartmans and de Bont 1992; Mattes et al. 2010; Vercé et al. 2000).

PCE is present in all of the source area locations identified at the industrial site. PCE is also present in the downgradient plume, within which the groundwater is predominantly aerobic (based on the presence of dissolved oxygen, elevated oxidation-reduction potential, and a lack of electron donor). Consequently, site-wide use of bioremediation will only be feasible for PCE if the areas that are aerobic are driven to anaerobic conditions, e.g., through addition of an organic electron donor or oxygen scavenger; bioaugmentation may also be required to achieve adequate rates of reductive dechlorination. Once PCE and TCE are removed under anaerobic conditions, a switch to aerobic conditions may be necessary to facilitate removal of residual cDCE and VC. Experimental studies with samples from the site were used to evaluate the presence of microbes capable of aerobically biodegrading cDCE and VC. As discussed further below, a switch to aerobic conditions will likely be necessary, at some point, to achieve efficient removal of benzene and CB.

In the event that biostimulation and/or bioaugmentation are not sufficient alone to achieve an adequate rate of dechlorination, introduction of zero valent iron (ZVI) could be useful and compatible with subsequent polishing by bioremediation. Such polishing may be required to remove low levels of chlorinated ethenes that may persist after exposure to ZVI. PCE levels in the source zones are sufficiently high (i.e., in the tens of milligrams per liter) to warrant consideration of using ZVI, followed by bioremediation.

In cases where redox conditions are high ZVI can also consume electron acceptors such as oxygen. One of the objectives of this study was to determine the rate of chlorinated ethene dechlorination using ZVI, and if the use of ZVI poses any challenges to follow-up use of bioremediation for polishing purposes.

1.2 Chlorinated Benzenes and Benzene

Under anaerobic conditions, reductive dechlorination of 1,2,4-TCB proceeds through 1,4-DCB to CB, which tends to accumulate. Reduction of CB to benzene has generally been regarded as an insignificant process (Beurskens et al. 1994). However, Fung et al. (2009) demonstrated reductive dechlorination of CB to benzene in sediments from a historically CB contaminated site, and suggested that the process occurs via organohalide respiration. As with any form of hydrogenolysis, an electron donor is required before dechlorination and biostimulation can be used for process enhancement. Lactate, glucose, ethanol, methanol, propionate, acetate, and hydrogen have been tested successfully (Middeldorp et al. 1997). 1,2-DCB and 1,3-DCB also undergo reductive dechlorination to CB (Bosma et al. 1988). The presence of DCB isomers, CB, and benzene at the industrial site suggests that microbes capable of reductively dechlorinating 1,2,4-TCB are present. One of the objectives of this study was to evaluate reductive dechlorination of chlorinated benzenes at the site, including the impact that biostimulation may have on the rate of dechlorination.

Biodegradation of benzene has been reported under several anaerobic electron accepting conditions, including nitrate-reducing (Chakraborty and Coates 2005; Kasai et al. 2007), iron-reducing (Anderson et al. 1998; Lovley et al. 1996), sulfidogenic

(Anderson and Lovley 2000; Mancini et al. 2003), and fermentative/methanogenic (Liang et al. 2013). Recently, Liang et al. (2013) described an intriguing consortium in which benzene is fermented to acetate and hydrogen under methanogenic conditions, with subsequent use of the hydrogen as an electron donor for reductive dechlorination of CB to benzene. One of the objectives of this study was to determine if microbes capable of reductively dechlorinating CB to benzene and anaerobically metabolizing benzene are present at the industrial site.

Under aerobic conditions, 1,2,4-TCB can be used as a sole carbon and energy source by *Pseudomonas* sp. strain P51, resulting in complete mineralization (van der Meer et al. 1991). The same microbe also grows on 1,2-DCB and 1,4-DCB (van der Meer et al. 1991). Elango et al. (2011) describe a *Variovorax* sp. that grows on 1,2-DCB as its sole source of carbon and energy and also cometabolizes cDCE and TCE. Microbes that use CB and benzene as sole carbon and energy sources under aerobic conditions are ubiquitous in soils (Kurt and Spain 2013). One of the objectives of this study was to evaluate the presence of these types of microbes at the industrial site.

Although there is mounting evidence in support of anaerobic biodegradation of benzene, this process appears to be atypical and commercial bioaugmentation cultures for enhancing activity are not currently available. Likewise, although reductive dechlorination of CB to benzene is known to occur, this activity also appears to be atypical. If these processes turn out to be atypical for the industrial site, then implementation of bioremediation will require a switch from anaerobic to aerobic conditions, since it was considered more likely that aerobes capable of biodegrading CB

and benzene are present. There is ample evidence to indicate that aerobes capable of biodegrading benzene, CB and DCBs are capable of surviving for extended periods under anaerobic conditions (Elango 2010). Once aerobic conditions are established, these microbes tend to respond quickly.

Engineering sequential anaerobic/aerobic conditions *in situ* is challenging; nevertheless, it has been demonstrated. For example, Beeman and Bleckmann (2002) used this approach to treat a plume of PCE and benzene in an aquifer underlying a closed and capped industrial landfill at the DuPont Plant near Victoria, TX. Perhaps most relevant to the industrial site, Burns et al. (2013) showed that reductive dechlorination of 1,2,4-TCB under anaerobic conditions occurred concurrently with microaerophilic oxidation of DCBs and CB. This suggests it may be feasible to add low amounts of air to anaerobic environments in which reductive dechlorination is occurring, yet still achieve aerobic oxidation of the aromatic contaminants. The success of this type of activity is related to the extremely high affinity that many oxygenases have for oxygen, permitting use of oxygen even at virtually non-detectable concentrations. This phenomenon has been demonstrated for microaerophiles that grow aerobically on VC (Gossett 2010) and likely applies to microbes that grow on aromatic compounds. One of the objectives of this study was to evaluate this concept in order to assess the feasibility of achieving concurrent anaerobic and aerobic conditions *in situ*.

1.3 Objectives

The overall objective of this laboratory study was to determine if bioremediation and/or abiotic degradation can be used to remediate the various contaminated areas at the industrial site. The specific objectives of the laboratory study were:

1) To determine the effectiveness of anaerobic biostimulation with lactate and emulsified vegetable oil (EVO) for the four source locations of contamination at the site, as well as the downgradient plume;

2) To determine the effectiveness of anaerobic biostimulation with lactate followed by aerobic biodegradation of any remaining contaminants, for the four source locations of contamination at the site, as well as the downgradient plume;

3) To determine the effectiveness of bioaugmentation to remove the chlorinated ethenes under anaerobic conditions, for the four source locations of contamination at the site, as well as the downgradient plume;

4) To determine the effectiveness of bioaugmentation to remove the chlorinated ethenes under anaerobic conditions followed by aerobic biodegradation of any remaining contaminants, for the four source locations of contamination at the site, as well as the downgradient plume;

5) To determine the effectiveness of removing contaminants with ZVI and biostimulation with lactate under anaerobic conditions for the four source locations of contamination at the site (ZVI was not considered for the downgradient plume on the basis of lower expected concentrations of contaminants); and

6) To determine the effectiveness of removing contaminants with ZVI and biostimulation with lactate under anaerobic conditions followed by aerobic biodegradation of any remaining contaminants, for the four source locations of contamination at the site.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Sample Locations

Groundwater and soil cores were collected by TRC Environmental Corporation on January 30 and 31, 2014. Groundwater was collected from wells at each source location as well as downgradient of the sources plumes. A total of 3 L was collected for each location, in three 1 L amber glass bottles. Soil cores were collected at each of these locations, below the water table, in 1.5 inch plastic sleeves, ranging in length from approximately 3 to 5 feet. This spanned a sufficient depth to provide a representative sample. Upon arrival at Clemson University, the groundwater and soil cores were stored at 4 °C, until the microcosms were prepared.

2.2 Chemicals and Medium

The types and sources of chemicals used for this project are summarized in Table 2.1. An aerobic enrichment culture was developed by transferring an aliquot from a microcosm to a mineral salts medium (MSM). An anaerobic MSM was also created in order to evaluate the effect of chlorinated benzenes and benzene on the performance of a chlorinated ethene bioaugmentation culture. The components of the anaerobic MSM are listed in Table 2.2 and the processes used to prepare both types of media are described in Appendix A.

2.3 Experimental Design

The experimental design is outlined in Table 2.3. A total of 147 microcosms were constructed. Justification for each treatment is provided below.

Unamended anaerobic microcosms simulated current *in situ* conditions. Unamended aerobic-only microcosms were used to evaluate the presence of microbes capable of biodegrading the lesser chlorinated compounds and the monoaromatic compounds.

Biostimulation was evaluated with lactate and EVO as electron donors. Biostimulation treatments with lactate were also evaluated for sequential anaerobic/aerobic conditions.

Bioaugmentation was evaluated for each location, using the KB-1 culture from SiREM and lactate as the electron donor. A bioaugmentation treatment was also evaluated for sequential anaerobic/aerobic conditions.

The effectiveness of ZVI was evaluated only with the source zone locations. Lactate was also added to these microcosms. A ZVI/lactate treatment was also evaluated for sequential anaerobic/aerobic conditions.

Autoclaved controls were prepared for each location, to account for differences in types of soils present. Autoclaved controls simulated the adsorptive losses within the microcosms. One set of water controls was prepared, containing concentrations relevant to each compound present in the microcosms. Water controls were prepared to determine the extent of diffusive losses from the microcosms.

2.4 Microcosm Preparation and Operation

2.4.1 Preparation

The day prior to preparing the microcosms, 1 L of groundwater from each location was moved from storage (4°C) to the anaerobic chamber, allowed to warm overnight poured into a 2 L media bottle, and resazurin was added (1 mg/L). Resazurin is a colorimetric redox indicator that turns from pink to colorless below an E_h of -110 mV (Jacob 1970). Potentials below this level are considered conducive to reductive dechlorination. Resazurin is a common component of anaerobic mineral medium used to cultivate strict anaerobes in the laboratory. It has subsequently been used in numerous microcosm studies because it permits a visual indicator of the redox level in the bottle, without having to remove a sample and perform a more complex E_h measurement.

After adding resazurin, the media bottle was capped and transferred to the anaerobic chamber, where it was allowed to warm overnight to room temperature. The soil cores were cut into 18 inch lengths (outside the laboratory, due to significant release of volatile compounds during cutting) and temporarily sealed with Parafilm. Using a steel rod and a spoon (both of which were sterilized prior to use for soil from each location), the soil was discharged into sterile Tupperware containers and then aggressively mixed with the spoon in order to homogenize it. The soil was then immediately added to the microcosms. Afterwards the containers were sealed to reduce water loss and loss of volatile organics (although organics can adsorb to plastics).

The anaerobic microcosms were prepared inside the anaerobic chamber by adding 20 ± 0.2 g of soil and 50 mL of groundwater to 160 mL serum bottles, leaving

approximately 99mL of headspace, and sealing them temporarily with slotted grey butyl rubber septa. Once all of the bottles for one location were ready, they were removed from the anaerobic chamber. The headspace of each bottle was sparged for 1 min with N₂ to remove the hydrogen present in the atmosphere of the anaerobic chamber (~1-5%) and then the bottles were sealed with Teflon-faced butyl rubber septa.

After allowing the headspace and liquid phases to reach equilibrium, a headspace sample from one of the triplicate bottles from each treatment was analyzed by gas chromatography (GC) (see below). The average level of PCE, TCE, 1,2,4-TCB, 1,2-DCB, 1,4-DCB, CB and benzene present was then compared to the target level for each contaminant. Target ranges are summarized in Table 2.4. The lower end of the range is based on the geometric mean for that compound and the upper end is the maximum concentration detected, over several years of field monitoring data (provided by TRC). Based on the average initial concentration in the microcosms and the target levels, a plan was developed to boost the concentration of contaminants to within these target ranges. The plan is summarized in Table 2.5. If increasing the amount of compound involved adding less than 1 µL of the neat compound, then it was added as a water-saturated solution. Consequently, only PCE and CB were added to the tank farm microcosms as neat compounds. Water-saturated solutions were prepared by equilibrating an excess of neat compound in contact with groundwater (so that a non-aqueous phase was present along with the water) for at least one week in a sealed bottle, to allow the compound to saturate the water. Because of the low solubility of the compounds in water, significant

volumes of the water-saturated solutions were needed. An upper limit on the amount to add was set at 10 mL per bottle.

Once the total volumes to add were calculated, the bottles were returned to the anaerobic chamber and the soil was allowed to settle. Caps were then removed one at a time, the requisite volume of groundwater was removed using an Eppendorf pipette (Table 2.5). For treatments with ZVI, 0.2 g was added at this time. The bottles were once again sealed temporarily with slotted gray butyl rubber septa.

Next, the bottles were removed from the anaerobic chamber and purged a second time for 1 min with N₂. For the tank farm and WWTP microcosms, the saturated solutions of PCE, 1,2,4-TCB, and 1,2-DCB were added to the microcosms just before the purging was stopped and the bottles were sealed. The reason for doing so was because the volume to add (3.0-4.5 mL per compound) was high enough that doing so with the bottles sealed would have over-pressurized them. Once these bottles were sealed, the remaining water-saturated solutions and neat compounds were injected by syringe. For the grease trap, ORP and downgradient microcosms, the bottles were sealed after sparging with N₂ and the required amounts of water-saturated solutions (≤ 2.5 mL/bottle) were injected. Finally, after allowing the liquid phase and headspace to equilibrate again for at least 4 h, a time zero measurement was made for all of the serum bottles based on analysis of headspace samples.

The aerobic microcosms were prepared in the same manner, under anaerobic conditions. Thereafter, 5.0 mL of pure oxygen was injected into the headspace, yielding

a partial pressure of approximately 5%. A lower than atmospheric concentration of oxygen was used to better simulate microaerobic conditions.

Autoclaved controls were prepared anaerobically as described above, except that they were autoclaved for 1 h on three consecutive days. Since autoclaving destroyed most of the contaminants, it was necessary to add back the chlorinated ethenes, chlorinated benzenes, and benzene, once the autoclaving process was completed. This returned the chlorinated ethenes, chlorinated benzenes, and benzene to the target concentrations.

Water controls consisted of each of the target compounds (1,2,4-TCB, DCB isomers, CB, benzene, PCE, TCE, cDCE, and VC), plus a volume of glass beads that displaced the same volume of water as the soil added to the live microcosms. This ensured that the water controls had the same ratio of headspace to liquid as the microcosms. The water controls were prepared on the bench top and therefore with room air in the headspace, but were sparged with nitrogen before capping.

When not being monitored, the bottles were placed in an inverted position (liquid and soil in contact with the septum) inside boxes and stored under quiescent conditions, at room temperature. Quiescent conditions were used for all microcosms to simulate the lack of turbulence in situ. The aerobic treatments, autoclaved controls, and water controls were stored on the bench top; all other treatments were stored in the anaerobic chamber.

2.4.2 Operation

The initial amounts of electron donor added were based on achieving a 10 fold excess of the hydrogen needed for complete dechlorination. Hydrogen yields were based on calculations reported in *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents* (30). EVO was added in the form of a commercial product called Anaerobic BioChem (ABC®), which is a mixture of glycerol (51%), C₁₈ fatty acids (9%) and ethyl lactate (0.5%). The estimated hydrogen yields per mole of substrate were 2 for lactate, 3 for glycerol, and 16 for fatty acids. Acetate was assumed not to undergo anaerobic oxidation and therefore no hydrogen yield was ascribed to it. Stock solutions of lactate were prepared such that each dose was delivered via an injection ranging from 0.025 mL (for the downgradient microcosms) to 1.0 mL (for the grease trap microcosms). ABC® was injected as received, at dosing ranging from 0.005 mL (for the downgradient microcosms) to 0.30 mL (for the grease trap microcosms). Additional amounts were added periodically in an effort to promote or sustain reductive dechlorination. Electron donor additions are denoted on the figures for each set of microcosms.

The initial pH of the groundwater from the WWTP, ORP, and downgradient was 6.8, 7.2, and 6.8, respectively. No adjustment was made. The pH was checked at least one time during operation and it remained in the circumneutral range (6.5-7.5), so buffer addition was not necessary. With the tank farm and the grease trap, the initial pH levels were low enough (6.2 and 6.5, respectively) such that the groundwater pH was adjusted with EOS buffer prior to setting up the microcosms. During incubation, the pH level

decreased below 6.5 and 100 μL of buffer was added to the Tank Farm microcosms. Unfortunately this was too high a dose and caused the pH to increase above 8. The pH was subsequently readjusted to circumneutral by gradually adding 1 M HCl. Based on this experience, only 50 μL of buffer was added to the grease trap microcosms and that increased the pH into the circumneutral range.

2.5 Analytical Procedures

1,2,4-TCB, 1,2-DCB, 1,4-DCB, CB, and benzene were analyzed by injecting 500 μL of headspace samples onto a HP 5890 Series II Plus GC equipped with an RTX 5 column (30-m \times 0.53-mm \times 1.5- μm film; Restek Corp.) and flame ionization detector (Elango et al. 2010). The injector and detector temperatures were 250°C and 325°C, respectively. The oven temperature program was 50°C for 4 min, rise at 10°C per min to 80°C, hold 10 min, rise at 10°C per min to 150°C, and hold for 1 min. Hydrogen (5 mL/min) was used as the carrier gas and nitrogen (30 mL/min) served as the makeup gas. The elution times were 3.3 min for benzene, 7.9 min for CB, 15.3 min for 1,4-DCB, 17.1 min for 1,2-DCB, and 22.6 min for 1,2,4-TCB. The standard curves for 1,2,4-TCB, 1,2-DCB, 1,3-DCB, 1,4-DCB, CB and benzene on this chromatograph are presented in Appendix B

PCE, TCE, cDCE, VC, ethane, ethene and methane were analyzed by injecting 500 μL of a headspace sample onto a GC (HP 5890 Series II) equipped with a 60/80 Carbowax B column (Supelco) and flame ionization detector, as previously described (Freedman and Gossett 1989). The GC response to a headspace sample was calibrated to

give the total mass of compound (M) in that bottle (Gossett 1987). The standard curves for PCE, TCE, cDCE, VC ethane, ethene, and methane are presented in Appendix B.

Assuming the headspace and aqueous phases were in equilibrium, the total mass present was converted to an aqueous phase concentration (equation 1):

$$C_l = \frac{M \times M_w}{(V_l + V_g H_c) \times 1000} \quad (1)$$

Where C_l = concentration in the aqueous phase (mg/L); M = total mass present ($\mu\text{mol/bottle}$); M_w = molecular weight of the compound ($\mu\text{g}/\mu\text{mol}$); V_l = volume of the liquid in the bottle (L); V_g = volume of the headspace in the bottle (L); H_c = Henry's constant ($((\text{mol}\cdot\text{m}^{-3} \text{ gas concentration})/(\text{mol}\cdot\text{m}^{-3} \text{ aqueous concentration}))$) at 23°C, and 1000 is to convert μg to mg. Table 2.6 lists the values for the Henry's constants for each compound.

The microcosm results presented in this report are in terms of μmol per bottle. This approach affords an opportunity to directly observe the stoichiometry of reductive dechlorination. However, it does not communicate the aqueous phase concentrations. Table 2.6 lists the constants for converting from M to C_l , based on equation 1. The conversion factors range from 0.42 for VC to 3.1 for 1,2,4-TCB. For example, a microcosm with 1.0 μmol per bottle of VC or 1,2,4-TCB would have aqueous phase concentrations of 0.42 and 3.1 mg/L, respectively.

Oxygen was analyzed by injecting 500 μL of a headspace sample onto a GC (HP 5890 Series II) equipped with thermal conductivity detector and molecular sieve 5A 60/80 column (1.8 m \times 3.1 mm; Alltech) (Elango et al. 2010). The detector, oven, and

injector temperatures were set at 120, 70 and 120°C, respectively. Nitrogen (30 mL/min) was used as the reference gas and carrier gas. The elution time for oxygen was 3.79 min. Room air was used to develop a response factor (i.e., percent oxygen per GC peak area unit). Since the detector response is linear over the range that was tested (i.e., 0-21% O₂), a one point calibration was considered acceptable. Response factors are presented in Appendix B.

Dehalococcoides and the three genes central to organohalide respiration of chlorinated ethenes (i.e., *tceA*, *vcrA*, and *bvcA*) were quantified at time zero for each of the source areas (4 samples) by Microbial Insights, based on 1 L samples of groundwater. After approximately one year of incubation, samples from 12 anaerobic microcosms that were biostimulated with lactate were sacrificed for in-house analysis of the same parameters for each of the source areas (4 treatments x triplicate bottles per treatment). Bacteria were collected by centrifuging the groundwater from each microcosm at 4°C, 4000g for 10 min on an Eppendorf centrifuge 5804R. The liquid was poured off and DNA was extracted from the pellets using a PowerSoil® DNA Isolation Kit (Catalog #12888-50) following the manufacturer's protocol (MO BIO Laboratories). Total numbers of *Dehalococcoides* 16S rRNA, *tceA*, *bvcA*, and *vcrA* genes were quantified using previously described primers and probes (Ritalahti et al. 2006) and temperature program (Ritalahti and Löffler 2010). The qPCR reaction mix was prepared as shown in Table 2.7. Standard DNA was synthesized using the gBlock® Gene Fragments tool from IDT, which produced double stranded oligos that carried the target *Dehalococcoides* 16S

rRNA, *tceA*, *bvcA*, and *vcrA* genes. Fluorescence quantification was performed on an Applied Biosystems® StepOnePlus™ Real-Time PCR System.

CHAPTER THREE

3.0 RESULTS

Water controls were prepared to evaluate the extent of diffusive losses. Results for 1,2,4-TCB, 1,2-DCB, 1,4-DCB, CB, benzene, PCE and TCE are shown in Figure 3.1. The percent losses were higher for the aromatic compounds than PCE and TCE. While significant, the losses need to be viewed in the context of the extended incubation time of 337-423 days and the similar extent of losses from the autoclaved controls (described below). Most importantly, the results need to be interpreted in light of complete removal of the contaminants in a number of the live treatments (also described below).

Results for the microcosms are grouped with respect to location and type of treatment. At all of the locations, the concentrations of PCE and TCE were high enough to be readily quantified by headspace analysis. For the aromatic compounds, all of the locations had concentrations of at least one compound above 4 mg/L. The results for these compounds are described below. Lower concentrations of other aromatic compounds were also detected, but at levels that made quantification by headspace analysis more challenging. These results are reported in Appendix C, but are not described in the sections below.

3.1 Wastewater Treatment Plant

3.1.1 Autoclaved Controls

Results for the WWTP autoclaved controls are shown in Figure 3.2. Following 497 days of incubation, benzene did not decrease and the other aromatic compounds decreased by 21-27%; PCE and TCE each decreased by 56%. Much of the decrease occurred in the first several months, as might be expected due to adsorption. Overall, these decreases were similar to the water controls.

3.1.2 Anaerobic Only Treatments

In comparison to the autoclaved controls, there was no appreciable decrease in 1,2-DCB, CB, benzene, PCE or TCE in the unamended WWTP microcosms (Figure 3.3). Low levels of cDCE, VC and ethene were detected early in the incubation; this may have been a consequence of setting up the microcosms in the anaerobic chamber. Most importantly, there was no increasing or decreasing trend for these daughter products. Likewise, there was no trend towards an increase in methane. The results indicate that the contaminants persisted in the unamended anaerobic microcosms, and the soil and groundwater are likely deficient in readily biodegradable organic matter.

1,2-DCB decreased by 85% in the lactate-amended WWTP microcosms (Figure 3.4a), compared to 21% in the autoclaved controls. There was no appreciable decrease in CB or benzene. By contrast, complete dechlorination of PCE and TCE to ethene occurred, with transient accumulation of cDCE and VC. Methane started to accumulate after most of the VC was dechlorinated. These results indicate that the WWTP location contains an indigenous population of PCE and TCE dechlorinating microbes, including

Dehalococcoides. All that was lacking to promote complete dechlorination of the chlorinated ethenes was addition of a readily fermentable substrate. However, microbes capable of reductively dechlorinating 1,2-DCB and CB do not appear to be present.

The EVO-amended microcosms behaved similarly (Figure 3.5). There was a 72% and 25% decrease in 1,2-DCB and benzene, respectively, but no apparent decrease in CB. PCE and TCE were completely dechlorinated to ethene following 356 days of incubation, with transient accumulation of cDCE and VC. This result indicates that the WWTP location contains microbes capable of fermenting vegetable oil and producing hydrogen, which is the required electron donor for *Dehalococcoides*.

The bioaugmented microcosms received lactate as the electron donor, in the same amounts as the lactate-amended bottles. KB-1 was added on day 132 (Figure 3.6). This significantly accelerated the rate of PCE and TCE reduction to ethene, with dechlorination complete approximately 100 days sooner than in the lactate-amended treatment. 1,2-DCB and benzene decreased by 89% and 35%, while CB persisted. The KB-1 culture does not contain microbes capable of respiring chlorinated benzenes.

In the WWTP anaerobic microcosms amended with ZVI and lactate (Figure 3.7), reduction of 1,2-DCB was nearly complete (97%). As in the biostimulated treatments, benzene decreased (35%) and CB persisted. Dechlorination of PCE and TCE was complete by day 83. Also, ethane was a significant product along with ethene. Methane output was highest in this anaerobic treatment. These results indicate that use of ZVI along with lactate significantly accelerated dechlorination of PCE and TCE in comparison to lactate alone.

3.1.3 Aerobic Only Treatment

In comparison to the autoclaved controls, 1,2-DCB, CB and benzene decreased rapidly in the aerobic treatment (Figure 3.8). Similar results were obtained for 1,2,4-TCB and 1,4-DCB, the initial concentrations of which were lower by one order of magnitude (Appendix C). To confirm the apparent aerobic biodegradation activity, the bottles were respiked five times with each aromatic compound. This was accomplished by opening the microcosms briefly, removing groundwater, and adding an equivalent volume of saturated groundwater solutions containing each aromatic compound. The bottles were then resealed. Subsequent rates of aromatic compound removal were considerably higher than for the initial incubation, suggesting that the microbes responsible were increasing in numbers by using the contaminants as growth substrates. The step decreases in PCE and TCE were a consequence of volatilization while the bottles were opened to resupply the aromatic compounds; PCE and TCE were not respiked.

On several occasions, samples from these aerobic-only microcosms were used to bioaugment other microcosms, mainly for aerobic treatments for the other locations. In addition, samples from these bottles were used to start an enrichment culture that was provided with 1,2,4-TCB, 1,2-DCB, 1,4-DCB, CB and benzene (see below). By day 326, bottle #2 no longer had enough groundwater remaining, so monitoring was discontinued.

These results indicate that the WWTP location contains microbes that are capable of aerobically biodegrading the aromatic compounds that were tested. All that was lacking was to supply an adequate level of oxygen. This is noteworthy since the same

location also harbors indigenous microbes capable of completely dechlorinating PCE and TCE to ethene under obligate anaerobic conditions.

3.1.4 Anaerobic Followed by Aerobic Treatments

With three additional treatments, incubation under anaerobic conditions was followed by a switch to aerobic conditions. In the treatment that was amended with lactate, the results were similar to what was observed in the corresponding anaerobic only treatment, i.e., PCE and TCE were completely dechlorinated to ethene by day 207 (Figure 3.9). Several days later, oxygen was added to the headspace of the bottles in order to switch to aerobic conditions. Over the next six months, there was a steady rate of decrease in 1,2-DCB, CB and benzene, commensurate with a steady rate of oxygen consumption (data not shown). To confirm that the disappearance of the aromatic compounds was biotic, the microcosms were respiked with the aromatic compounds on day 414. A higher rate of removal occurred which validates the growth of microbes that metabolize the aromatic compounds. In addition to the aromatic compounds, ethene was also consumed after the microcosms were converted to aerobic conditions.

Similar results were obtained in the bottles that were amended with lactate and bioaugmented with KB-1 (Figure 3.10), and those that were treated under anaerobic conditions with ZVI and lactate (Figure 3.11). After dechlorinating the PCE and TCE to ethene (and ethane in the ZVI bottles) and switching to aerobic conditions, the aromatic compounds were consumed at a steady rate. The rate of aromatic compound removal was higher after respiking, indicative of growth. In the ZVI amended bottles, aerobic consumption of ethane is apparent, along with ethene. Figure 3.11 is the average for the

triplicate bottles. In one of these, consumption of the aromatic compounds continued to near completion by day 500. It is unclear what caused the rate of benzene and CB biodegradation to slow down in the other two bottles. Oxygen was not limiting; when levels in the headspace decreased below 1%, pure oxygen was added to restore the level to 5%.

The anaerobic/aerobic results indicate that the aerobic microbes responsible for consumption of the aromatic compounds at the WWTP location are able to tolerate extended periods of incubation under the low redox conditions necessary for anaerobic reductive dechlorination of PCE and TCE. Once they were switched to aerobic conditions, a steady rate of consumption of the aromatic compounds occurred, which was well above any decreases that occurred over the same time interval in the autoclaved controls.

3.2 Oil Retention Pond

3.2.1 Autoclaved Controls

Results for the ORP autoclaved controls are shown in Figure 3.12. Following 495 days of incubation, CB and benzene decreased by 62% and 46%, respectively. There was a slight net decrease in PCE (13%) and TCE (5%), although there was an increase between day 0 and 17 (possibly due to inadequate equilibration at time zero), followed by a slow decline. Much of the decrease in aromatics occurred in the first several months, as might be expected due to adsorption. Overall, the magnitude of change in the autoclaved controls was similar to the water controls.

3.2.2 Anaerobic Only Treatments

In comparison to the autoclaved controls, there was no appreciable decrease in CB, benzene, PCE or TCE in the unamended ORP microcosms (Figure 3.13). There was no trend towards an increase in methane, suggesting the absence of readily biodegradable electron donors.

In the lactate-amended microcosms, there was variability among the replicates with respect to PCE and TCE dechlorination. In microcosm #1, PCE and TCE were completely converted to ethene by day 404 (Figure 3.14b). In microcosm #2, dechlorination of PCE and TCE was well underway, with VC and ethene being the main products when monitoring was stopped (Figure 3.14d). In microcosm #3, PCE and TCE dechlorination had proceeded mainly through cDCE (Figure 3.14f), although the trend suggests complete dechlorination should be achievable. These results indicate that the ORP location contains an indigenous population of PCE and TCE dechlorinating microbes, including *Dehalococcoides*. All that was lacking to promote dechlorination of the chlorinated ethenes was addition of a readily fermentable substrate, although slow rates of dechlorination may make this approach undesirable. Over the same time interval, there was no apparent trend in anaerobic biodegradation of CB or benzene (Figure 3.14a, c, e) in comparison to the autoclaved controls.

Unlike lactate, addition of EVO did not stimulate reductive dechlorination of PCE and TCE (Figure 3.15). There was a significant increase in methane beginning around day 193, indicating that microbes capable of fermenting vegetable oil are present in the ORP soil and groundwater. It is unclear why EVO was a successful substrate for the

WWTP location but not the ORP. Although unclear, differences in the microbial community may affect the fermentation of EVO and cause hydrogen to not be produced. Over the 354 day incubation period, there was no appreciable decrease in CB or benzene in comparison to the autoclaved controls.

The bioaugmented microcosms received lactate as the electron donor, in the same amounts as the lactate-amended bottles. KB-1 was added on day 130 (Figure 3.16). This significantly accelerated the rate of PCE and TCE reduction to ethene, with dechlorination complete approximately 50 days following bioaugmentation. In spite of the active dechlorination of PCE and TCE, CB and benzene persisted.

In the ORP anaerobic microcosms amended with ZVI and lactate (Figure 3.17), reduction of CB was significant (82%) compared to the autoclaved controls (62%). Benzene behaved similarly to CB, with a decrease of 69% compared to 46% in the autoclaved controls. The decrease in the aromatic compounds was likely due to adsorption. Dechlorination of PCE and TCE was complete by day 50 and ethane was the principal end product. These results indicate that use of ZVI along with lactate significantly accelerated dechlorination of PCE and TCE in comparison to lactate alone.

3.2.3 Aerobic Only Treatment

Over the first 48 days of incubation in the aerobic-only microcosms, there was a faster rate of decrease in CB and benzene compared to the autoclaved controls, but then activity ceased (Figure 3.18). CB decreased by 83% in the aerobic microcosms versus 62% in autoclaved controls over the same interval; benzene decreased by 74% in the aerobic microcosms, compared to 45% in the controls. It is unclear why activity ceased

beyond day 48. On day 324, the aerobic-only ORP microcosms were bioaugmented with 1 mL from the aerobic-only WWTP microcosms (Figure 3.18); by day 395 there was no indication that this addition initiated aerobic biodegradation of CB and benzene. PCE and TCE did not change significantly compared to the autoclaved microcosms.

3.2.4 Anaerobic Followed by Aerobic Treatments

With three treatments, incubation under anaerobic conditions was followed by a switch to aerobic conditions. In the treatment that was amended with lactate, all of the PCE and TCE were consumed by day 242; by day 348, only a low level of VC remained, with ethene being the predominant product (Figure 3.19a). Over this same interval, the decreases in CB and benzene were of the same magnitude as in the autoclaved controls. The decision was made to switch to aerobic conditions on day 348. Over the next 76 days, there was no noticeable decrease in ethene; CB and benzene levels fluctuated, with no consistent trend indicative of biodegradation (Figure 3.19a). This confirmed the ORP aerobic-only microcosms, suggesting that the ORP location is lacking in a robust population of monoaromatic degrading aerobic microbes. The lactate-amended anaerobic/aerobic microcosms were bioaugmented on day 477 with samples from the WWTP aerobic microcosms. After bioaugmentation, most all of the CB was consumed, and there were notable decreases in benzene, ethene, and methane.

The microcosms that were bioaugmented with KB-1 achieved complete reduction of PCE and TCE to ethene by day 220, at which point oxygen was added (Figure 3.20). Thereafter, CB and benzene started to decrease consistently, albeit at a modest rate. On day 324, 1 mL from the aerobic-only WWTP microcosms was added and this

significantly accelerated the rate of CB and benzene biodegradation, such that their removal was complete by day 367. Similar results were observed with 1,2-DCB and 1,4-DCB, which were present at lower concentrations (Appendix C). The aromatic compounds were added again on day 368 and the subsequent rate of biodegradation was considerably faster, with complete removal by day 457. These results confirmed the potential to use anaerobic followed by aerobic conditions to remove the chlorinated ethenes and aromatic compounds at the ORP location. Given the slow rate of activity with the indigenous aerobic microbes at this location, bioaugmentation with a culture from the WWTP location appears to be warranted.

In the microcosms amended with ZVI and lactate, PCE and TCE were removed at a much faster rate than with lactate alone and with bioaugmentation (Figure 3.21). These bottles were switched to aerobic conditions on day 220 and a sample from the aerobic-only WWTP microcosms was added. However, CB and benzene persisted. On day 324, another dose was made from the aerobic-only WWTP microcosms and this time there was a rapid response. This was likely a consequence of building up the population of aromatic degrading microbes in the aerobic WWTP microcosms via repeated respiking. Complete removal of CB and benzene was achieved by day 367. Both compounds were respiked and a faster rate of aerobic biodegradation ensued, with removal completed by day 495. A lower dose of 1,2- and 1,4-DCB was made at the same time, and both were biodegraded by day 410 (Appendix C).

These anaerobic/aerobic results indicate that addition of soil and groundwater from the WWTP site can be used to increase the rate of aerobic biodegradation of

aromatics at the ORP location. Following bioaugmentation with soil and groundwater from the WWTP aerobic-only microcosms, a steady rate of consumption of the aromatic compounds occurred. The rate and extent of activity significantly exceeded the loss of these compounds from the autoclaved controls over the same time interval.

3.3 Tank Farm

3.3.1 Autoclaved Controls

Results for the tank farm autoclaved controls are shown in Figure 3.22. Following 483 days of incubation, the aromatic compounds decreased by 0-34%. PCE and TCE decreased by 14% and 23% respectively. Much of the decrease in aromatics occurred in the first several months, as might be expected due to adsorption. Overall, these decreases were similar to the water controls.

3.3.2 Anaerobic Only Treatments

In comparison to the autoclaved controls, there was no appreciable decrease in 1,2-DCB, CB, benzene, PCE or TCE in the unamended tank farm microcosms (Figure 3.23). Neither daughter products nor methane formed in this treatment. The results indicate that the contaminants persisted in the unamended anaerobic microcosms.

There was no appreciable decrease in 1,2-DCB, CB, benzene, PCE, or TCE in the tank farm microcosms that were amended with lactate (Figure 3.24), amended with EVO (Figure 3.25), or amended with lactate and bioaugmented (Figure 3.26). Based on the lack of response to the initial addition of KB-1 on day 136, a second dose was added on day 242, to no avail. More experiments on the tank farm are required to determine why the bioaugmented microcosms did not respond as the WWTP and ORP responded to

bioaugmentation. The pH of the microcosms was adjusted to 7.3 to 7.8, which is appropriate for KB-1. The initial concentration of PCE, TCE, CB and benzene in the tank farm microcosms was on the order of 5 to 10 fold higher than in the WWTP and ORP microcosms. Information on the inhibitory effect of CB and benzene on the performance of KB-1 was not found. An inhibition experiment was subsequently conducted with levels similar to the tank farm (section 3.8).

In the tank farm anaerobic microcosms amended with ZVI and lactate (Figure 3.27), there was a significant decrease in PCE (96%) and TCE (91%) as well as benzene (31%), CB (43%), and 1,2-DCB (65%) over the 480 days of incubation. The main products from PCE and TCE were ethene and ethane; daughter products from the aromatic compounds were not detected. Adsorption of the aromatics to carbon in the ZVI (3% carbon content) may have played a role in their greater percent removal from the ZVI-amended microcosms in comparison to the autoclaved controls.

3.3.3 Aerobic Only Treatment

There was no appreciable decrease in PCE or TCE in the aerobic-only tank farm microcosms during 484 days of incubation (Figure 3.28), in comparison to the autoclaved controls. Through day 330, the magnitude of decrease in 1,2-DCB, CB and benzene was greater than in the autoclaved controls, although activity stalled after day 71. On day 330, 1 mL of sample from the aerobic-only WWTP microcosms was added to each Tank Farm aerobic bottle. This appeared to improve the extent of 1,2-DCB biodegradation, but not benzene or CB. On day 417, another dose from the aerobic-only WWTP microcosms was added, with more decreases occurring. A third addition was added on day 482;

continued monitoring is needed to ascertain if aerobic biodegradation is feasible in the presence of the high levels of PCE and TCE.

3.3.4 Anaerobic Followed by Aerobic Treatments

With three treatments (lactate-amended, bioaugmented, and ZVI and lactate-amended), the intent was to switch from anaerobic to aerobic conditions once the PCE and TCE were consumed. However, as noted above for the anaerobic-only treatments, PCE and TCE removal was incomplete in all of these treatments through 480 days of incubation (Figures 3.29, 3.30, and 3.31). Consequently, none of the bottles were switched to aerobic conditions. PCE and TCE persisted under anaerobic conditions even after two doses of KB-1 (Figure 3.30). In the bioaugmented bottles, corn syrup was added on day 177 in a further attempt to establish the low redox conditions needed for reductive dechlorination of PCE and TCE. Corn syrup was added due to the fact that a wider range of microbes can oxidize hexose to reduce various electron acceptors and eventually cause fermentation. Addition of corn syrup was also unsuccessful in stimulating reductive dechlorination.

In the tank farm treatment amended with ZVI and lactate (Figure 3.31), there was a significant decrease in PCE and TCE. There was no appreciable decrease in benzene and CB compared to the autoclaved controls. 1,2-DCB was reduced by 69% after 507 days of incubation. Adsorption of the aromatics to the ZVI may have played a role in the reduction of 1,2-DCB. Coupled with the reduction of PCE and TCE, there was a subsequent increase in ethene and ethane. Complete removal of PCE was reached after

507 days of incubation. This long period before complete dechlorination may be related to the high initial concentration of PCE and/or an inadequate initial dose of ZVI.

3.4 Grease Trap

3.4.1 Autoclaved Controls

Results for the grease trap autoclaved controls are shown in Figure 3.32. Following 482 days of incubation, 1,2,4-TCB, PCE, and TCE decreased by 46%, 18%, and 16%, respectively. Due to the comparatively low volatility of 1,2,4-TCB, fluctuations in the headspace measurement occurred. However these fluctuations do not affect the overall conclusions reached.. A peak corresponding to cDCE appeared on day 79 and then remained level; since there was no decrease in PCE or TCE, this compound was likely something other than cDCE that happened to coelute. The same peak also appeared in the live treatments.

3.4.2 Anaerobic Only Treatments

In comparison to the autoclaved controls, there was no appreciable decrease in 1,2,4-TCB, 1,2-DCB, PCE or TCE in the unamended anaerobic grease trap microcosms (Figure 3.33). No daughter products were detected and methane formation was not observed. These results indicate that the contaminants persisted in the unamended anaerobic microcosms.

Biostimulation with lactate did not result in any significant decrease in 1,2,4-TCB, 1,2-DCB, PCE or TCE in comparison to the autoclaved controls, through 407 days of incubation (Figure 3.34). There was also no net decrease in 1,2,4-TCB, 1,2-DCB, PCE or TCE in the EVO-amended microcosms (Figure 3.35). These results indicate that

the required microbes are absent and/or conditions for reductive dechlorination are unfavorable.

Bioaugmentation with KB-1 was also ineffective in establishing reductive dechlorination of PCE and TCE (Figure 3.36). Based on the lack of response to the initial addition of KB-1 on day 133, two doses of corn syrup were added (days 175 and 192) in an attempt to lower the redox level in the bottles and make the environment more conducive to reductive dechlorination. A second dose of KB-1 was added on day 255, all to no avail. It is not yet clear why the grease trap microcosms did not respond to bioaugmentation, as was observed with the WWTP and ORP locations. The pH of the microcosms was adjusted to 7.0 to 7.5, which is appropriate for KB-1. The initial concentration of PCE, 1,2,4-TCB and 1,2-DCB in the grease trap microcosms was several fold higher than in the WWTP and ORP microcosms; this may have created an inhibitory environment for KB-1. Information on the inhibitory effect of 1,2,4-TCB and 1,2-DCB on the performance of KB-1 was not found in the literature. A subsequent experiment to evaluate inhibition was conducted with concentrations similar to the grease trap. Results are presented in section 3.8.

In the grease trap anaerobic microcosms amended with ZVI and lactate, there was an appreciable decrease in PCE (70%) and TCE (62%) in comparison to the autoclaved controls; a modest decrease in 1,2,4-TCB, and 1,2-DCB occurred, but there was no increase in daughter products, suggesting that adsorption was responsible (Figure 3.37). The results suggest that ZVI was not effective at the dose tested for the grease trap location.

3.4.3 Aerobic Only Treatment

There was no appreciable decrease in 1,2,4-TCB, 1,2-DCB, PCE or TCE in the grease trap aerobic-only microcosms (Figure 3.38) when compared to the autoclaved controls. On days 328 and 415, the microcosms were amended with 1 mL from the aerobic-only WWTP microcosms. The first dose had no impact; the second dose was similarly ineffective.

3.4.4 Anaerobic Followed by Aerobic Treatments

With three treatments (lactate-amended, bioaugmented, and ZVI and lactate-amended), the intent was to switch from anaerobic to aerobic conditions once the PCE and TCE were consumed. However, as noted above for the anaerobic-only treatments, the extent of decrease in PCE and TCE in the lactate-amended (Figure 3.39) and bioaugmented microcosms (Figure 3.40) was similar to what occurred in the autoclaved control bottles, and there was no accumulation of daughter products. As described above, the bioaugmented bottles received two doses of corn syrup and well as two doses of KB-1, without effect. Consequently, both treatments were never switched to aerobic conditions. 1,2,4-TCB and 1,2-DCB also persisted, through 499 days of incubation. As mentioned above, the lack of anaerobic activity may be related to high initial concentrations of PCE, TCE, 1,2,4-TCB and 1,2-DCB.

In the grease trap treatment amended with ZVI and lactate (Figure 3.41), PCE decreased by approximately 35%, commensurate with accumulation of ethene and ethane. There was no significant decrease in TCE. The slow rate of PCE dechlorination over the 499 days of incubation was likely related to too low a dose of ZVI in comparison

to the high initial concentrations of contaminants. 1,2-DCB decreased in these microcosms by approximately 30%, which was notably higher than in the autoclaved controls (no appreciable loss). Nevertheless, the lack of daughter products (CB or benzene due to reductive dechlorination) suggests the decrease was attributable to adsorption associated with carbon in the ZVI. Since PCE and TCE persisted in these bottles, they were never switched to aerobic conditions.

3.4.5 Soil Analysis

A soil sample from the grease trap location was evaluated by the Clemson University Agricultural Service Laboratory, which uses the Mehlich-1 extraction procedure (0.05 N HCl and 0.25 N H₂SO₄). Results are shown in Table 3.1. All of the constituents were below 300 ppm, suggesting that the lack of biological activity in the grease trap microcosm was not a consequence of inhibition related to metals. Manganese was present at >269 ppm. Since Mn⁴⁺ can serve as an electron acceptor and thereby create a demand for electron donor, it may have had an impact on establishing conditions favorable to reductive dechlorination. However, as will be discussed below, the amount of donor added was well in excess of the demand that was likely exerted by manganese.

3.5 Downgradient

3.5.1 Autoclaved Controls

Results for the downgradient autoclaved controls are shown in Figure 3.42. Following 493 days of incubation, the aromatic compounds decreased by 37-66%. PCE and TCE decreased by 41% and 45%, respectively. There was no increase in the daughter products or change in the concentration of methane that would indicate biotic activity.

Much of the decrease in aromatics occurred in the first several months, as might be expected due to adsorption. Overall, these decreases were similar to the water controls. The comparatively large error bars are related to the lower concentration of contaminants present in the downgradient microcosms; lower concentrations generally result in greater variability in quantification. A small amount of oxygen entering the microcosm could cause degradation of chlorinated benzenes. Although no corresponding shift in color (from clear to pink) occurred.

3.5.2 Anaerobic Only Treatments

In the unamended anaerobic treatment for the downgradient microcosms, there was no apparent decrease in PCE or TCE in comparison to the autoclaved controls, and there was no significant accumulation of daughter products (Figure 3.43). However, 1,2-DCB decreased below detection by the end of the incubation period (day 493), while CB and benzene decreased by 99% and 61%, respectively. Lower losses occurred in the autoclaved controls, suggesting that the decrease in aromatic compounds was a biotic process. Nevertheless, as 1,2-DCB decreased there was no corresponding transient increase in CB or benzene, as would be expected with reductive dechlorination. It is possible that over the nearly 500 days of incubation, small amounts of oxygen diffused into the bottles during measurements, resulting in aerobic oxidation of the aromatic compounds.

There was no appreciable reductive dechlorination of PCE or TCE in the lactate-amended downgradient microcosms (Figure 3.44). Decreases in 1,2-DCB, CB and benzene were of a similar magnitude to the autoclaved controls, suggesting that the

microbes needed for dechlorination of chlorinated ethenes and benzenes are lacking in the downgradient location. Similar results were obtained for the EVO-amended microcosms (Figure 3.45).

The microcosms that were bioaugmented with KB-1 received lactate as the electron donor, in the same amounts as the lactate-amended bottles (Figure 3.46). KB-1 was added on day 128. This resulted in rapid reduction of PCE and TCE to cDCE by day 156. However, activity stalled at cDCE, such that a second dose of KB-1 was added on day 252. This initiated rapid reduction of cDCE to VC and a slower accumulation of ethene. As of the last day of sampling (day 493), reduction to ethene was nearly complete. Bioaugmentation with KB-1 did not have a discernable effect on removal of 1,2-DCB, CB or benzene, all of which persisted following an initial decrease over the 493 days of incubation.

3.5.3 Aerobic Only Treatment

Two of the three downgradient aerobic-only bottles exhibited high rates of 1,2-DCB, CB, and benzene biodegradation, with complete removal by day 52 (Figure 3.47c, e); the third bottle also exhibited biodegradation activity, although it stalled after day 52 (Figure 3.47a). All of the bottles were respiked on day 169 with 1,2-DCB, CB, and benzene, followed by a higher rate of biodegradation in two of the three bottles (Figure 3.47c, e), consistent with the likelihood that microbes were using the aromatic compounds as growth substrates. It is unclear why one of the bottles lagged behind the other two. 1,2,4-TCB was present at approximately a five-fold lower concentration; it too was biodegraded below detection in the two most active bottles (Appendix C).

Overall, these results indicate that the downgradient location contains microbes capable of aerobically biodegrading 1,2-DCB, CB, and benzene. During the 428 days of incubation, PCE and TCE persisted. The perturbations around day 169 were a consequence of respiking, during which time the samples needed to regain equilibrium. To prevent volatile losses as occurred in the WWTP-AER bottles, the soil in these bottles was allowed to settle and a needle was inserted through the soil and into the water, which was withdrawn. This was done in lieu of opening the bottles. This limited the losses in chlorinated ethenes that occurred in WWTP aerobic microcosms

3.5.4 Anaerobic Followed by Aerobic Treatments

With two treatments (lactate-amended and bioaugmented), the intent was to switch from anaerobic to aerobic conditions once the PCE and TCE were consumed. However, the lactate-amended bottles did not respond to the electron donor addition (Figure 3.48), as described above for the lactate-amended anaerobic treatment. PCE and TCE persisted, without accumulation of daughter products. Consequently, these bottles were not switched to aerobic conditions. 1,2-DCB, CB and benzene persisted in these microcosms over the 365 days of incubation.

In the bioaugmented treatment, KB-1 was added on day 128 (Figure 3.49). As in the bioaugmented bottles described above, PCE and TCE were rapidly reduced to cDCE, but then dechlorination stalled. A second dose of KB-1 was made on day 252 and by day 325, ethene was the predominant product, with a trace level of VC remaining (~0.1 $\mu\text{mol/bottle}$). At that time, the bottles were switched to aerobic conditions and they were further bioaugmented with samples (2 mL per bottle) from the aerobic-only WWTP

microcosms. CB subsequently decreased at the highest rate, followed by benzene and 1,2-DCB. On day 366, each compound was respiked; by day 408, all were consumed below detection. During the aerobic period of incubation, the remaining low level of VC was consumed, as was most of the accumulated ethene. These results indicate it is possible to achieve anaerobic reductive dechlorination of PCE and TCE to ethene in the downgradient location, based on bioaugmentation with KB-1. Furthermore, it is then possible to switch to aerobic conditions and accomplish complete biodegradation of the aromatic compounds. Bioaugmentation with an aerobic culture derived from the WWTP location made it possible to achieve removal of the aromatic compounds at a higher rate than what would likely have occurred using only the indigenous aromatic degraders.

3.6 qPCR Estimation of *Dehalococcoides* and Halorespiratory Genes

Groundwater samples from the WWTP, ORP, tank farm and grease trap locations were sent to Microbial Insights for estimation of *Dehalococcoides* and three halorespiratory genes (*tceA*, *bvcA*, and *vcrA*). At the end of the incubation period, the lactate-amended anaerobic microcosms for the same locations were sacrificed for in-house analysis of the same parameters. The results are summarized in Table 3.2. Only the WWTP groundwater tested positively for *Dehalococcoides*, *bvcA*, and *vcrA*. This is consistent with the subsequent results from the microcosms, i.e., the highest rates of PCE and TCE dechlorination occurred in the WWTP microcosms. The final levels of *Dehalococcoides*, *bvcA*, and *vcrA* were higher, as would be expected after stimulating dechlorination activity by providing lactate as the electron donor. It is unclear why *tceA*

did not increase; it is conceivable that a related enzyme was involved but was not amplified by the *tceA* primers used.

Comparable levels of *Dehalococcoides*, *bvcA*, and *vcrA* were also detected in the ORP lactate-amended anaerobic microcosms; this too was consistent with the significant stimulation of dechlorination activity in these microcosms by addition of lactate. An increase in the *tceA* gene was also observed in these microcosms. The results confirm the physiological observation that indigenous dechlorinators are present at the WWTP and ORP but are currently lacking in electron donor.

In the tank farm and grease trap anaerobic microcosms amended with lactate, very low levels of *Dehalococcoides* were detected. Similarly low levels of *tceA* and *vcrA* were detected in the grease trap microcosm. These levels are very close to the quantification limit and do not provide compelling evidence for the presence of indigenous chloroethene respiring microbes. This is consistent with the fact dechlorination of PCE and TCE did not occur in any of the biostimulated microcosms from these locations. Furthermore, even bioaugmentation was ineffective, indicating that the conditions at both locations are unfavorable for the growth of these types of microbes.

3.7 Aerobic Enrichment Culture

An aerobic enrichment culture was created from the WWTP aerobic microcosms. The enrichment was prepared with MSM (Appendix A) under aerobic conditions. The monoaromatic compounds were added to triplicate serum bottles using water-saturated solutions: 4 mL of 1,2,4-TCB; 1.2 mL of 1,2 DCB; 1 mL of 1,4 DCB; 0.6 mL of CB; and 0.2 mL benzene. As seen in Figure 3.50, the aerobic enrichment culture readily degraded

the first and a second addition of the aromatic compounds. For the third addition, the amount of 1,2,4-TCB was increased by adding 1 μ L of neat compound, rather than adding a large amount of saturated water solution; the other compounds were added as saturated water solutions. The intent was to achieve a 1,2,4-TCB that is closer to the levels found in the grease trap location (Table 2.4). However, the increase in 1,2,4-TCB was inhibitory; the rate of biodegradation decreased substantially for all of the monoaromatics. In an effort to revive the enrichment, more inoculum was added from the WWTP aerobic microcosms on days 64 and 78. Approximately one month later, activity resumed at a high rate and the four compounds were consumed by day 141, when a fourth spike of 1,2,4-TCB, 1,2-DCB, 1,4-DCB, CB and benzene was added. A high rate of consumption ensued, with complete removal by day 154. These results indicate that it will be feasible to develop an aerobic enrichment culture using inoculum from the WWTP location, for the purpose of bioaugmenting the ORP and downgradient locations and thereby enhance aerobic biodegradation of the aromatic contaminants.

3.8 Inhibition

In spite of bioaugmenting the tank farm and grease trap microcosms with KB-1 and lactate, there was no response in anaerobic reductive dechlorination of PCE and TCE. One possible explanation for this is inhibition of the KB-1 culture by the high levels of aromatic compounds found at both locations. To evaluate this hypothesis, an experiment was prepared with three treatments: PCE + TCE alone (at the average levels found at the tank farm and grease trap locations), PCE + TCE + monoaromatic compounds found at the same level as in the tank farm microcosms; and PCE + TCE +

monoaromatic compounds found at the same level as in grease trap microcosms. Only anaerobic MSM was used (50 mL/bottle); glass beads were added to displace a volume equivalent to the soil used in the microcosms. To hasten the results of the experiment, ten times the volume of KB-1 culture that was added to the microcosms was added for this experiment (0.5 mL). Each treatment consisted of triplicate bottles. PCE was added as a neat compound, while the other compounds were added using saturated solutions in MSM. MSM was used instead of water due to the high volumes of saturated solutions needed (20.3 mL/bottle for the tank farm and 38 mL/bottle for the grease trap); adding water-saturated solutions risked diluting the MSM and making it difficult to maintain low redox conditions.

Results for the treatment with only PCE and TCE added are shown in Figure 3.51. Stoichiometric reduction to ethene was observed by day 30. This indicated that the level of PCE and TCE found in tank farm and grease trap microcosms was not the reason for a lack of dechlorination activity.

Figure 3.52a shows the concentration of aromatic compounds in the treatment that contained contaminant levels similar to the tank farm microcosms. There were no losses, consistent with the expectation that KB-1 is not intended for chlorinated aromatic compounds. In Figure 3.52b it can be seen that PCE and TCE were consumed by day 42, with nearly stoichiometric accumulation of ethene. A low level of VC remained that likely would be reduced with additional incubation. Although the rate of dechlorination was somewhat lower than in the absence of the monoaromatics, these results indicate that

the levels of these compounds in the tank farm microcosms was not the reason for a lack of activity on PCE and TCE.

In the treatment with monoaromatics present at levels found in the grease trap microcosms, the levels of 1,2,4-TCB and 1,2-DCB were stable during the 31 days of incubation (Figure 3.53a). However, these levels were sufficient to prevent reductive dechlorination of PCE and TCE (Figure 3.53b). It is unclear if the inhibition was caused by one or the other or both. Since 1,2-DCB was present at levels similar to what was used in the tank farm treatment (Figure 3.52a), it seems likely that 1,2,4-TCB is responsible for inhibiting KB-1. Regardless, this result helps to explain the lack of reductive dechlorination activity for PCE and TCE in the grease trap microcosms.

3.9 Summary

Table 3.3 summarizes the microcosm results for this study. Sequential anaerobic followed by aerobic treatment was effective for removing the chlorinated ethenes and aromatic compounds in microcosms prepared with samples from the WWTP, ORP and downgradient locations of the site. An evaluation of the results from each location is provided below.

3.9.1 Wastewater Treatment Plant

Anaerobic biostimulation with lactate or EVO was sufficient to dechlorinate PCE and TCE in the WWTP microcosms. This is consistent with the presence of indigenous halorespiring microbes that are currently limited by a lack of electron donor. For the WWTP microcosms, bioaugmentation with KB-1 was not required, but it did accelerate

the rate of dechlorination. Bioaugmentation in the field is worthy of consideration based on the increased rate of chlorinated ethene dehalogenation observed in the microcosms.

Treatment with ZVI achieved dechlorination of PCE and TCE at the highest rate and resulted in ethene and ethane as the terminal products. However, it is less likely that use of ZVI for the WWTP is worthwhile based on significant challenges associated with deployment.. Challenges include the labor intensive process of implementing a permeable reactive barrier by digging a trench that reaches the aquitard, or the high pressure required to inject solids into the subsurface. The aromatic compounds decreased significantly in the ZVI-amended WWTP treatments, although this was likely due to adsorption. Unlike PCE and TCE, daughter products from the chlorinated benzenes did not accumulate as the concentration of aromatic compounds decreased.

There was little evidence of anaerobic dechlorination of the aromatic compounds in the WWTP microcosms. However, this location contains a robust population of aerobic microbes capable of biodegrading the aromatics. This was confirmed when repeat additions of the aromatics were consumed at a faster rate than the initial dose in the aerobic-only microcosms. Of perhaps greatest significance, the aerobic bacteria survived an extended period under anaerobic conditions in the anaerobic/aerobic microcosms, while PCE and TCE were being dechlorinated to ethene. Once oxygen was added, the aerobes initiated a steady rate of aromatic degradation. The rate of degradation was enhanced by bioaugmenting with samples from the WWTP aerobic-only microcosms, which presumably became enriched in aromatic degraders after consuming several additions of the aromatic compounds.

One of the challenges posed by sequential anaerobic/aerobic treatment is the oxygen demand left behind from excess electron donor applied during anaerobic treatment. Careful monitoring of electron donor is advisable to avoid an excess, so that when the environment is switched to aerobic conditions, there is not an excessive oxygen demand associated with the readily degradable donor and its fermentation products. One way to avoid this problem is to use sequential aerobic/anaerobic treatment. This was not evaluated as part of the scope of this microcosm evaluation, but is worthy of consideration. By starting out under aerobic conditions, the oxygen demand will be limited to the aromatic compounds. Once those are removed, conditions can be turned anaerobic. While this may negatively impact the indigenous population of anaerobic halo-respiring bacteria, bioaugmentation with a commercially available culture can be used to compensate.

Although an indigenous population of aerobic aromatic degrading microbes is present at the WWTP location, results indicated that adding samples from the aerobic-only microcosms accelerated the rate of aerobic biodegradation following extended periods of time under anaerobic conditions. One way to make optimum use of the indigenous aromatic degraders at the WWTP location would be to cultivate them in an on-site aerobic reactor. By feeding the reactor with contaminated groundwater from the WWTP location and possibly the more contaminated tank farm and grease trap groundwaters, indigenous aromatic degraders can be grown on the aromatics at the site. The bacteria grown in the reactor could then be used as an enrichment culture to bioaugment areas during aerobic remediation. Another approach would be to send

samples from the WWTP location to a vendor that specializes in developing enrichment cultures. The vendor would then return it to the site for deployment once it has reached a high concentration of aromatic degraders.

All of the aromatic compounds decreased under anaerobic conditions, but this also occurred in the autoclaved controls, indicating that adsorption was significant. However, as Table 3.3 indicates, there was substantial anaerobic biodegradation of 1,2-DCB in the bioaugmented and ZVI treatments, based on a greater extent of decrease in comparison to the killed controls. Reductive dechlorination seemed to be an unlikely explanation for degradation of the 1,2-DCB, since during the time when it was decreasing, CB and benzene did not increase. Anaerobic oxidation of monoaromatic compounds is possible, but further study would be required to conclusively determine what mechanism was at work in this study.

3.9.2 Oil Retention Pond

Anaerobic biostimulation with lactate was sufficient to dechlorinate PCE and TCE in the ORP microcosms; EVO was not an effective electron donor. As with the WWTP microcosms, bioaugmentation with KB-1 accelerated the rate of PCE and TCE dechlorination. ZVI was also effective in dechlorinating PCE and TCE at a high rate. The ORP location is lacking in indigenous aerobic aromatic degraders. Consequently, the aerobic part of sequential anaerobic/aerobic treatment was accomplished in all three sequential treatments by adding samples from the aerobic-only WWTP microcosms. Thus, sequential anaerobic/aerobic treatment appears to be a viable strategy for the ORP location with the addition of an aerobic enrichment culture.

3.9.3 Tank Farm and Grease Trap

The tank farm and grease trap locations were the most challenging for bioremediation. Reductive dechlorination of PCE and TCE was not achieved, even with KB-1 bioaugmentation. Results from the inhibition experiment indicate that the levels of benzene, CB, and 1,2-DCB in the tank farm microcosms had only a slight inhibitory effect on reductive dechlorination of PCE and TCE, indicating that some other factor was responsible for the inhibition. However, the high level of 1,2,4-TCB in the grease trap microcosms was inhibitory to KB-1 (Figure 3.53). Analysis of the grease trap soil suggest that metals did not contribute to the inhibition (Table 3.1).

KB-1 inhibition in the grease trap microcosms is consistent with another behavior of these microcosms, i.e., the difficulty in establishing low redox conditions. This was indicated by the groundwater remaining pink, based on the color of the resazurin that was added to the groundwater. Pink indicates the redox level was above -110 mV; a level lower than that is generally consider necessary for growth of chloroethene respiring microbes. Establishment of low redox conditions was reached within several weeks of preparing the WWTP, ORP, tank farm, and downgradient microcosms. More than sufficient lactate and EVO was added to the grease trap microcosms to lower the redox level; nevertheless, the pink color persisted. The first addition of KB-1 was made when the groundwater was pink (since it was being added to all of the other bioaugmentation bottles), although this likely inactivated the culture. In an attempt to remedy the elevated redox problem, corn syrup was added twice. Hexose is more readily fermented by a

wider variety of microbes than lactate and EVO. This approach was effective in lowering the redox level and subsequently a second addition of KB-1 was made, but to no avail.

Even though the redox level did eventually drop, this experience suggests that the grease trap contains contaminants or minerals that are inhibitory to the establishment of low redox conditions. Analysis of a soil sample by the Clemson University Agricultural Service Laboratory indicated that all of the minerals analyzed were below 100 ppm, with the exception of manganese, which was present at >269 ppm. The quantity of manganese present was higher than the detection limit of the soil analysis, so the actual concentration may be higher. Manganese dioxide (Mn^{4+}) can serve as a terminal electron acceptor, at a higher redox level than chlorinated organics, thereby interfering with reductive dechlorination. Assuming the manganese content of the soil is 300 ppm and all of it is bioavailable, the electron donor demand for manganese reduction in 20 g of soil is 0.22 meq per bottle. Table 3.4 shows that the total amount of electron donor added to treatments that exhibited reductive dechlorination of PCE and TCE (WWTP, ORP and downgradient) ranged from 0.77 to 2.66 meq per bottle, well in excess of the hypothesized Mn^{4+} demand. Furthermore, the fact that reductive dechlorination was achieved in these treatments demonstrates that manganese was not inhibitory. Thus, in spite of the high level of manganese in the site soil, it was not likely the reason for the lack of PCE and TCE dechlorination in the tank farm and grease trap locations.

Elevated levels of sulfate may also inhibit reductive dechlorination. Data for sulfate levels in the groundwater was not available. However, sulfate levels were measured at the conclusion of the incubation time in one of the three bottles for each

treatment (with the exception of anaerobic lactate amended bottles, which were sacrificed for qPCR analysis). As shown in Table 3.4, the concentrations in the grease trap, WWTP, ORP and downgradient microcosms were below 10 mg/L. Although initial groundwater sulfate concentrations were not measured, sulfate likely did not change in the aerobic-only treatments, in which sulfate reduction did not occur. There were no substantial differences between the final levels measured in the aerobic-only and anaerobic treatments, suggesting that sulfate reduction was a minor process in these microcosms. Sulfate levels were higher in the tank farm microcosms (up to 115 mg/L). This was a consequence of a pilot scale evaluation of in situ chemical oxidation using persulfate, approximately 7 years prior. Nevertheless, even for the highest sulfate level measured, the electron donor demand for sulfate reduction amounted to only 0.48 meq per bottle, compared to addition of 16 to 29 meq per bottle of electron donor to the tank farm microcosms (Table 3.4). Thus, it appears unlikely that sulfate was responsible for inhibition of reductive dechlorination of PCE and TCE in the tank farm microcosms.

During headspace analysis of the grease trap microcosms, several peaks eluted whose identity is unknown. It is conceivable that one or more of these compounds is contributing to inhibition of reductive dechlorination by KB-1. Identification of these compounds might reveal other contaminants that contribute to inhibition.

A complication of note with the tank farm microcosms involved pH adjustment. Since the pH of the groundwater was 6.2 and the optimum pH for dehalorespiration is approximately 6.5 to 7.5, the decision was made to add a buffer to all of the biotic microcosms. A preliminary test with only groundwater suggested that a dose of 100 μ L

should be sufficient. However, after adding that dose and rechecking the microcosms, the pH rose above 8, to as high as 9.9. Excessively high pH is also detrimental to dechlorinators, so it was necessary to readjust by lowering the pH with 1 M HCl. This was done gradually (over several days) to avoid overshooting the circumneutral range. Nevertheless, it is not known if this excursion into a high pH range adversely impacted subsequent bioaugmentation with KB-1.

Perhaps the most surprising result with the tank farm and grease trap microcosms was the lack of significant activity in the treatments amended with ZVI. Since this is an abiotic process, it should not be subjected to the same limitations experienced with bioaugmentation. Nevertheless, the rate of PCE and TCE dechlorination was much slower than in the WWTP and ORP microcosms amended with ZVI added. This suggested that the ZVI dose in the tank farm and grease trap microcosms was too low for the amount of PCE and TCE present. An experiment was subsequently performed in separately prepared microcosms from both locations, which included a ZVI dose that was five times higher than what was used to prepare the original set of bottles. Immediately after ZVI addition dechlorination began and daughter products started to accumulate (data not shown). These results suggest that ZVI treatment may be feasible for the grease trap and tank farm locations, as long as an adequate dose is determined. The feasibility of then switching to aerobic conditions to treat the aromatic compounds would still need to be determined.

3.9.4 Downgradient

Complete treatment of the chlorinated ethenes and the aromatics in the downgradient microcosms was achievable by sequential anaerobic/aerobic treatment. Reductive dechlorination of PCE and TCE was possible only with bioaugmentation; indigenous halorespiring microbes are lacking at this location. One issue of concern was that dechlorination stalled at cDCE after the first addition of KB-1, for reasons that are not yet understood. After a second addition of KB-1 to reestablish reductive dechlorination, activity was restored, although at a slower rate than may be anticipated.

Two of the three aerobic-only microcosms exhibited significant rates of aromatic biodegradation; it is unclear why the third microcosm lagged behind, as they were prepared in an identical manner. The anaerobic/aerobic bioaugmented treatment responded quickly to addition of a sample from the aerobic-only WWTP microcosms, suggesting that bioaugmentation with an aerobic aromatic enrichment culture would be also advisable for the downgradient location, following anaerobic removal of the chlorinated ethenes.

CHAPTER FOUR

4.0 DISCUSSION

4.1 Effect of Hydrology on Bioremediation

Hydrology plays an important role in bioremediation. Based on the types of soils involved at the site evaluated in this research, the permeability and therefore flowrate of groundwater can be greatly affected. The aquifer at the industrial site is described as fractured bedrock covered by regolith (USGS 2014). The regolith contains mostly saprolite which is typically considered to be clayey. Clays and bedrock tend to have low permeability. In a system such as this, groundwater flow will follow the fractures in the rock formations. Water may also flow through a few other areas that contain more sandy deposits. The aquifer description indicates that pockets of high permeability allow groundwater to flow through the site. However, the majority of the area contains low permeability clays that restrict groundwater flow. In this case, the low permeability areas increase the difficulty of distributing the electron donors and cultures required for remediation.

Low permeability can also cause contaminants to follow the fissures that exist in the bedrock, causing a unique contaminant plume shape. The permeability of an area may also allow diffusion of contaminants into the clayey sediments, which could then desorb over time, preventing quick elimination of the contaminants. Since the USGS report spoke in generalities for a multi-state region, it is prudent that a hydrogeological survey for the specific area be used before finalizing any decisions on remediation.

4.2 Remediation Techniques

If increasing the local permeability of a site is required, then it may be accomplished by causing fractures in either the bedrock or in the clay soils. In addition to increasing the permeability of the area, reactive materials may also be included in the supportive matrix of the created fractures, improving the reaction rates. In field experiments by Siegrist et al. (1998), horizontal fractures were made by boring to specified depths, applying a high pressure water jet to cut a horizontal notch in the soil, and introducing injection fluid into the borehole at a constant rate. Pressure quickly exceeding a critical value of the clays will cause a fracture to form. The injection material may contain coarse sands, guar gum gel, and possibly a reactive material. In a field scale experiment, permanganate was used as a reactive material; a special grout was needed due to the potential for oxidation by permanganate (Siegrist et al. 1998). This delivery strategy can also be used with ZVI. However, it is unclear if an adequate dose of ZVI could be delivered to address the high concentrations of chlorinated organics found in the tank farm and grease trap locations.

In the WWTP, ORP, and downgradient locations, the microcosm results indicate that sequential anaerobic/aerobic bioremediation is a viable strategy. The two ways of implementing sequential anaerobic/aerobic bioremediation are either temporally or spatially. In an area where contaminants are sequestered in a specific area, a temporal sequence is preferable. The plume is contained within the specific treatment area while injection wells are used to add and distribute the electron donor required for reductive

dechlorination. The wells can later be used for addition of oxygen, so that aerobic biodegradation can occur.

In areas where contaminants are mobile, spatial sequencing of anaerobic/aerobic conditions may be preferable. Injection wells can be used to add electron donor. Within the area designated for reductive degradation, the electron donor is spread via groundwater flow. Contaminants are typically encouraged to flow through this area by extraction wells or allowing natural flow to move groundwater downgradient to an aerobic zone (Beeman and Bleckmann 2002). A nutrient injection wall can also be formed. By injecting electron donor in pulses that correspond to groundwater flow, an area downgradient of the injection well continually receives donor (Devlin et al. 2004) for PCE removal. Monitoring of both the anaerobic and aerobic sections is required to ensure all contaminants are eliminated.

5.3 Amendments for Remediation

Remediation often entails adding materials to the subsurface. Typical amendments include organic electron donors, ZVI, or oxidative materials. Electron donors and ZVI (reducing agent) can be used together, but the addition of oxidizing agents (e.g., hydrogen peroxide, permanganate) in areas with electron donor or ZVI would be counterproductive by consuming each other as opposed to remediating the contaminant. This may also change local chemistry, further affecting biological remediation methods.

Electron donor doses varies greatly from site to site. In the conducted microcosms, the ratio of donor added to donor required for reductive dechlorination of

PCE and TCE to ethene is summarized in Table 5.1. Most of these ratios are range from 150 to 250. This is on the high end of ratios reported in other studies. For example, De Bruin et al. (1992) used a 150 fold excess. Lee et al. (1998) reported ratios ranging from 3.2 to 450,000, depending in part on the culture and donor evaluated. The culture that had a ratio of 450,000 was extremely inefficient and would not be used in any field applications.

Amendments such as ZVI, permanganate and hydrogen peroxide have been used to abiotically degrade contaminants in situ. ZVI uses reductive dechlorination to convert PCE and TCE to their daughter products. ZVI can be combined with other transition metals that can further increase its reactivity during dehalogenation by improving reaction kinetics and prevent surface corrosion (Fu et al. 2014). Iron is still oxidized, but the reactivity is mostly maintained. Hydrogen peroxide and permanganate can be used to oxidize PCE and TCE. Permanganate tends to transform TCE into various carboxylic acids (Yan and Schwartz 2000). The other products are chloride, manganese dioxide, and the cation that the permanganate was with (typically K^+). Oxidative conditions are unfavorable to anaerobic bioremediation of remaining PCE and TCE. However, oxidation can be effective for the removal of aromatic compounds (with the exclusion of permanganate). Starting the remediation process with aerobic degradation may prove beneficial if refractory compounds like 1,2,4-TCB inhibit anaerobic microbial processes. These refractory compounds may require persulfate or advanced oxidation such as Fenton oxidation (Huling and Pivetz 2006)

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the results of this microcosm study, the following conclusions are offered:

- 1) Sequential anaerobic/aerobic bioremediation is a viable treatment approach for the WWTP, ORP, and downgradient locations. PCE and TCE can be efficiently dechlorinated to ethene under anaerobic conditions and the aromatic compounds can be oxidized under subsequent aerobic conditions.
- 2) It may be feasible to use only anaerobic biostimulation to remove PCE and TCE for the WWTP and ORP locations, whereas bioaugmentation will be required for the downgradient location. Consideration should also be given to using bioaugmentation for the WWTP and ORP locations, as this will accelerate removal of PCE and TCE so that a switch to aerobic conditions can occur more quickly.
- 3) There was no compelling evidence in support of anaerobic biodegradation of the aromatic compounds, either via reductive dechlorination to benzene or via anaerobic oxidation. However, aerobic biodegradation of the aromatic compounds is feasible for the WWTP, ORP, and downgradient locations.
- 4) Development of an aerobic enrichment culture for biodegradation of the aromatic contaminants at the WWTP, ORP and downgradient locations is advisable. Since aerobic enrichment cultures typically are not commercially available, it is beneficial to produce one at the site. Aerobic aromatic degraders are present at the WWTP. Groundwater from

this location can be used to develop an indigenous bioaugmentation culture. The process of enrichment can either be done in an on-site reactor or by an off-site vendor. Bioaugmentation with an aerobic enrichment culture will significantly improve the rate of aerobic treatment of the aromatic compounds at the WWTP, ORP and downgradient locations.

5) Without additional evaluation, bioremediation alone is not feasible for the tank farm and grease trap locations. ZVI shows promise for treating the PCE and TCE, although additional study is warranted to find an appropriate dose. Also, additional studies are needed to ascertain if aerobic biological treatment would be feasible to address the aromatic compounds following ZVI treatment of PCE and TCE.

6) 1,2,4-TCB is at least partially responsible for the lack of PCE and TCE reductive dechlorination in the grease trap microcosms. This conclusion is based on the inhibition test, which showed that the presence of high concentrations of 1,2,4-TCB inhibited KB-1.

5.2 Recommendations

Recommendations for further research based on this study include:

1) Further studies of inhibition on chlorinated ethene degraders caused by chlorinated aromatics; specifically, evaluation of 1,2,4-TCB is needed to determine how the magnitude of inhibition varies with concentration.

2) Soil and water samples from the tank farm require further study to determine what inhibits the activity of KB-1. Analysis is required because there is currently no known reason for inhibition

- 3) Sequential aerobic/anaerobic conditions are worthy of evaluation. By using aerobic conditions first, the problems associated with increased oxygen demand associated with residual electron donor during anaerobic treatment are avoided. The survivability of indigenous halorespiring microbes to prolonged aerobic conditions needs to be determined.
- 4) Further experiments should be conducted to determine the effectiveness of aerobic removal of aromatics from the grease trap location, specifically 1,2,4-TCB, followed by anaerobic treatment.
- 5) The characteristics of the aerobic enrichment culture should be evaluated and compared to known monoaromatic degraders, e.g., *Pseudomonas* sp. strain P51 or *Bordetella petrii*, which is able to grow aerobically using 1,2,4-TCB as a sole source of carbon and energy.
- 6) Unknown peaks that appeared in the grease trap microcosms should be identified by GC/MS, and then evaluated for their contribution to inhibition of KB-1.
- 7) A remediation strategy involving several chemical oxidizing agents (e.g., permanganate, persulfate, Fenton oxidation) followed by bioremediation should be evaluated at the laboratory scale.
- 8) In future studies, GC response factors should be measured more frequently to provide more accurate analysis of the compounds measured.

TABLES

Table 2.1. Sources and purity of most chemicals used.

Chemical	Purity	Source
Sodium Lactate Syrup	60%	E. M. Scientific
EVO (ABC® = Anaerobic BioChem)	-	Redox Tech LLC
EOS CoBupH ^a	-	EOS
ZVI ^b	90%	Redox Tech LLC
PCE	99%	Acros
TCE	99%+	Acros
<i>cis</i> -1,2-DCE	99%	TCI
VC	>1 ppm nitrogen	Matheson
Ethene	99.50%	National Welders
Ethane	99.99%	Matheson
1,2,4-TCB	99%	Sigma Aldrich
1,2-DCB	99%	Fluka Analytical
1,3-DCB	99%	Aldrich
1,4-DCB	99%	Alfa Aesar
CB	99.90%	Sigma Aldrich
Benzene	100%	J.T. Baker
Methane	99.99%	Matheson

^a 45% alkaline solids, >1% dispersant, >0.5% stabilizer, balance = water.

^b 3% carbon.

Table 2.2. Components of the anaerobic MSM used in the inhibition test.

Chemical	Purity	Source
Aluminum Sulfate	98%	Mallinckrodt
Ammonium Chloride	99.5%	BDH
Bicarbonate	99%	Mallinckrodt
Boric Acid	99.5	EMD
Calcium Chloride	99%	EMD
Cobalt Chloride	99.8%	Mallinckrodt
Copper Chloride	99.8%	Sigma
Ferrous Chloride	99%	Mallinckrodt
Hydrochloric Acid	37%	Mallinckrodt
Magnesium Sulfate	98%	EMD
Magnesium Chloride	99%	Mallinckrodt
Nickel Chloride	98%	Mallinckrodt
Resazurin sodium derivative	NA ^a	JT Baker
Sodium Molybdate	98%	Fisher Scientific
Sodium Sulfide	60-63%	Acros Chemical
Sodium Selenate	98%	Sigma Aldrich
Yeast Extract	NA	Difco
Zinc Sulfate	99.3%	Fisher Scientific

^a Not available.

Table 2.3. Experimental design.^a

Treatment	Redox Condition(s)	Tank Farm	Grease Trap	WWTP	Oil Retention Pond	Downgradient Plume	Water Controls
As-is (unamended)	anaerobic only	3	3	3	3	3	-
As-is (unamended)	aerobic only	3	3	3	3	3	-
Biostimulation with lactate	anaerobic only	3	3	3	3	3	-
Biostimulation with EVO	anaerobic only	3	3	3	3	3	-
Bioaugmentation (with lactate)	anaerobic only	3	3	3	3	3	-
ZVI, followed by biostimulation (lactate)	anaerobic only	3	3	3	3	-	-
Biostimulation (lactate)	anaerobic/aerobic	3	3	3	3	3	-
Bioaugmentation (with lactate)	anaerobic/aerobic	3	3	3	3	3	-
ZVI, followed by biostimulation (lactate)	anaerobic/aerobic	3	3	3	3	-	-
Autoclaved controls	anaerobic	3	3	3	3	3	-
Water controls	-	-	-	-	-	-	3
Subtotals		30	30	30	30	24	3

^a Values in the table refer to the number of microcosms; triplicates were be prepared for each treatment and location.

Table 2.4. Targeted initial concentrations of contaminants based on historical monitoring data.

Compound	Target Initial Concentrations (mg/L)				
	Tank Farm	Grease Trap	WWTP	ORP	Downgradient Plume
PCE	40 - 120	10 - 60	8 - 23	0.5 - 2	0.1 - 0.3
TCE	2 - 11	1 - 6	1 - 3	0.2 - 3	0.1 - 0.2
1,2,4-TCB	2 - 7	2 - 33	2 - 4	0.1 - 0.5	0.05 - 0.2
1,2-DCB	16 - 50	5 - 14	2 - 3	0.1 - 0.5	0.4 - 1.4
1,4-DCB	2 - 15	1.5 - 5	1 - 3	0.1 - 0.8	0.1 - 0.2
CB	7 - 48	0.2 - 1.3	9 - 20	0.1 - 0.6	0.5 - 1
Benzene	2 - 13	0.01 - 0.1	0.5 - 6	0.1 - 0.6	0.03 - 0.1

Table 2.5. Volumes withdrawn and added to achieve targeted contaminant concentrations.

Removal or Addition	Volume Withdrawn or Added				Downgradient Plume
	Tank Farm	Grease Trap	WWTP	ORP	
<i>Volume removed (mL/bottle)</i>	10.0	1.0	10.0	2.5	1.6
<i>Compounds added (mL or μL/bottle)*</i>					
PCE	3.0 μ L	none	3.5 mL	1.0 mL	0.23 mL
TCE	0.5 mL	none	0.1 mL	0.1 mL	0.02 mL
1,2,4-TCB	3.0 mL	none	4.0 mL	0.2 mL	none
1,2-DCB	4.5 mL	none	1.2 mL	0.3 mL	0.6 mL
1,4-DCB	1.0 mL	1.0 mL	1.0 mL	0.4 mL	0.1 mL
CB	1.0 μ L	none	none	0.3 mL	0.6 mL
Benzene	1.0 mL	none	0.2 mL	0.2 mL	0.05 mL

* μ L amounts were added as neat compounds; mL amounts were added as saturated solutions in groundwater from the downgradient location.

Table 2.6. Factors to convert mass per bottle to aqueous phase concentrations.

Compound	H_c (23°C)	Conversion Factor
		(multiply μmol per bottle times this factor to obtain mg/L aqueous)*
1,2,4-TCB	0.08	3.1
1,2-DCB	0.07	2.6
1,3-DCB	0.13	2.3
1,4-DCB	0.12	2.4
CB	0.15	1.7
Benzene	0.22	1.1
PCE	0.64	1.5
TCE	0.35	1.6
cDCE	0.14	1.5
VC	1.01	0.42

* Based on a liquid volume of 50 mL, a headspace volume of 99 mL, 11 mL occupied by soil, molecular weight of the compound, and a temperature of 23°C.

Table 2.7. Components of qPCR reaction mix.^a

Component	Stock Solution	μL stock solution/ 25 μL reaction	Final Concentration
Master Mix ^b	2×	12.5	1×
Probe + primers ^c	6 mM	1.25	300 nM
DNA template	-	5.0	-
Water	-	6.25	-

^a Adapted from the MO BIO Laboratories Instruction Manual for PowerSoil® DNA Isolation Kit (Catalog #12888-50) and Ritalahti et al. (2006) to include a larger volume of DNA template but with a smaller total volume.

^b Taqman® Gene Expression Master Mix from ABI (Applied Biosystems).

^c Prober and primers are pre-mixed in a customized PrimeTime standard qPCR assay from IDT.

Table 3.1. Inorganic constituents in a soil sample from the grease trap location.

Constituent	Concentration (ppm)
P	21
K	25
Ca	49
Mg	>85
Zn	1.4
Cu	0.23
Mn	>269
Fe	22
Na	62
B	0.22
Al	13
As	<0.04
Cd	<0.04
Cr	<0.04
Mo	<0.12
Ni	0.14
Pb	<0.12
Se	<0.08

Table 3.2. qPCR results for groundwater and lactate-amended anaerobic microcosms.

Location	Sample Time	Sample Type	copies/mL*			
			<i>Dhc</i>	<i>tceA</i>	<i>bvcA</i>	<i>vcrA</i>
WWTP	Initial	Groundwater	6.7x10 ³	-	4.9x10 ³	1.8x10 ²
	Final	Microcosm†	10 ³ -10 ⁵	-	10 ² -10 ⁴	10 ³ -10 ⁵
ORP	Initial	Groundwater	-	-	-	-
	Final	Microcosm	10 ² -10 ⁶	10 ² -10 ⁶	10 ²	10 ² -10 ³
Tank Farm	Initial	Groundwater	-	-	-	-
	Final	Microcosm	10 ¹ -10 ²	-	-	-
Grease Trap	Initial	Groundwater	-	-	-	-
	Final	Microcosm	10 ¹ -10 ²	10 ²	-	10 ¹ -10 ²

* Results for the groundwater samples are based on extraction of 1 L, analyzed by Microbial Insights. For the microcosms, 25-37 mL was extracted from each bottle and analyzed in-house; results represent the range of values detected from duplicates or triplicate bottles; if only one value is reported, a positive response was obtained in only one bottle. The order of magnitude is reported for the microcosms rather more specific values based on the precision of the measurement from a relatively small sample size. A dash indicates below detection.

† Lactate-amended anaerobic microcosms.

Table 3.3. Summary of microcosm performance for each location and each treatment, with respect to the chlorinated ethenes (CE) and aromatic compounds (ARO).*

Treatment	Redox Condition(s)	WWTP		ORP		Tank Farm		Grease Trap		Downgradient	
		CE	ARO	CE	ARO	CE	ARO	CE	ARO	CE	ARO
As-is (unamended)	anaerobic only										(5)
As-is (unamended)	aerobic only						(2)				(6)
Biostimulation with lactate	anaerobic only										
Biostimulation with EVO	anaerobic only										
Bioaugmentation (with lactate)	anaerobic only		(1)							(7)	
ZVI, followed by biostimulation (lactate)	anaerobic only		(1)							N/A	N/A
Biostimulation (lactate)	anaerobic/aerobic										
Bioaugmentation (with lactate)	anaerobic/aerobic										
ZVI, followed by biostimulation (lactate)	anaerobic/aerobic					(3)		(4)		N/A	N/A

* A red cell indicates activity did not occur: a green cell indicates activity did occur in a reliable manner. N/A = not available. Numbers in a cell correspond to the following footnotes.

(1) Significant biodegradation of 1,2-DCB observed.

(2) Modest response on 1,2-DCB after bioaugmenting with the aerobic WWTP culture

(3) Most but not all of the PCE was consumed

(4) Partial dechlorination of PCE

(5) 1,2-DCB was consumed

(6) Two of three bottles were very active

(7) cDCE and VC are still present, but declining

Table 3.4. Sulfate concentrations in microcosms, including a comparison between electron donor required for reduction and electron donor added in each treatment.

Treatment	Sulfate			Donor added (meq/bottle)
	(mM)	(mg/L)	(meq/bottle)	
TF-ANA	0.82	78.7	0.33	-
TF-ANA-EVO	0.93	88.9	0.37	16
TF-ANA-AUG	1.19	114.5	0.48	29
TF-ANA-ZVI	0.74	71.4	0.30	16
TF-AER	0.14	13.9	0.06	-
TF-ANA/AER-LAC	1.05	100.6	0.42	16
TF-ANA/AER-AUG	1.03	98.6	0.41	29
TF-ANA/AER-ZVI	0.59	56.9	0.24	16
GT-ANA	0.06	6.2	0.03	-
GT-ANA-EVO	0.08	7.6	0.03	52
GT-ANA-AUG	0.05	5.1	0.02	120
GT-ANA-ZVI	0.04	3.5	0.02	60
GT-AER	0.06	5.4	0.02	-
GT-ANA/AER-LAC	0.04	3.9	0.02	60
GT-ANA/AER-AUG	0.04	3.7	0.02	120
GT-ANA/AER-ZVI	0.04	3.4	0.01	60
WWTP-ANA	0.00	0.4	0.00	-
WWTP-ANA-EVO	0.00	0.1	0.00	2.60
WWTP-ANA-AUG	0.00	0.1	0.00	1.93
WWTP-ANA-ZVI	0.00	0.1	0.00	1.93
WWTP-AER	0.00	0.4	0.00	-
WWTP-ANA/AER-LAC	0.00	0.3	0.00	1.93
WWTP-ANA/AER-AUG	0.00	0.2	0.00	1.93
WWTP-ANA/AER-ZVI	0.00	0.2	0.00	1.93
OR-ANA	0.02	2.0	0.01	-
OR-ANA-EVO	0.00	0.3	0.00	1.73
OR-ANA-AUG	0.00	0.3	0.00	1.29
OR-ANA-ZVI	0.00	0.5	0.00	0.77
OR-AER	0.00	0.4	0.00	-
OR-ANA/AER-LAC	0.00	0.3	0.00	1.03
OR-ANA/AER-AUG	0.01	0.5	0.00	1.03
OR-ANA/AER-ZVI	0.00	0.1	0.00	0.77
DG-ANA	0.01	0.5	0.00	-
DG-ANA-LAC	0.00	0.3	0.00	0.96
DG-ANA-EVO	0.00	0.1	0.00	1.30
DG-ANA-AUG	0.00	0.1	0.00	1.12
DG-AER	0.00	0.2	0.00	-
DG-ANA/AER-LAC	0.00	0.1	0.00	0.96
DG-ANA/AER-AUG	0.07	6.5	0.03	0.96

Table 5.1. Ratio of lactate added as donor to demand for PCE and TCE reduction to ethene.

Treatment	Required (meq/bottle)	Added (meq/bottle)	Ratio: Added/Required
WWTP-ANA-LAC	0.0419	5.79	138
WWTP-ANA-AUG	0.0317	5.79	182
WWTP-ANA-ZVI	0.3000	5.79	193
WWTP-ANA/AER-LAC	0.0322	5.79	180
WWTP-ANA/AER-AUG	0.0337	5.79	172
WWTP-ANA/AER-ZVI	0.0379	5.79	153
OR-ANA-LAC	0.0150	3.09	206
OR-ANA-AUG	0.0174	3.86	221
OR-ANA-ZVI	0.0058	2.31	401
OR-ANA/AER-LAC	0.0158	3.09	196
OR-ANA/AER-AUG	0.0153	3.09	201
OR-ANA/AER-ZVI	0.0053	2.31	436
DG-ANA-AUG	0.0035	3.38	977
DG-ANA/AER-AUG	0.0034	2.89	850

FIGURES

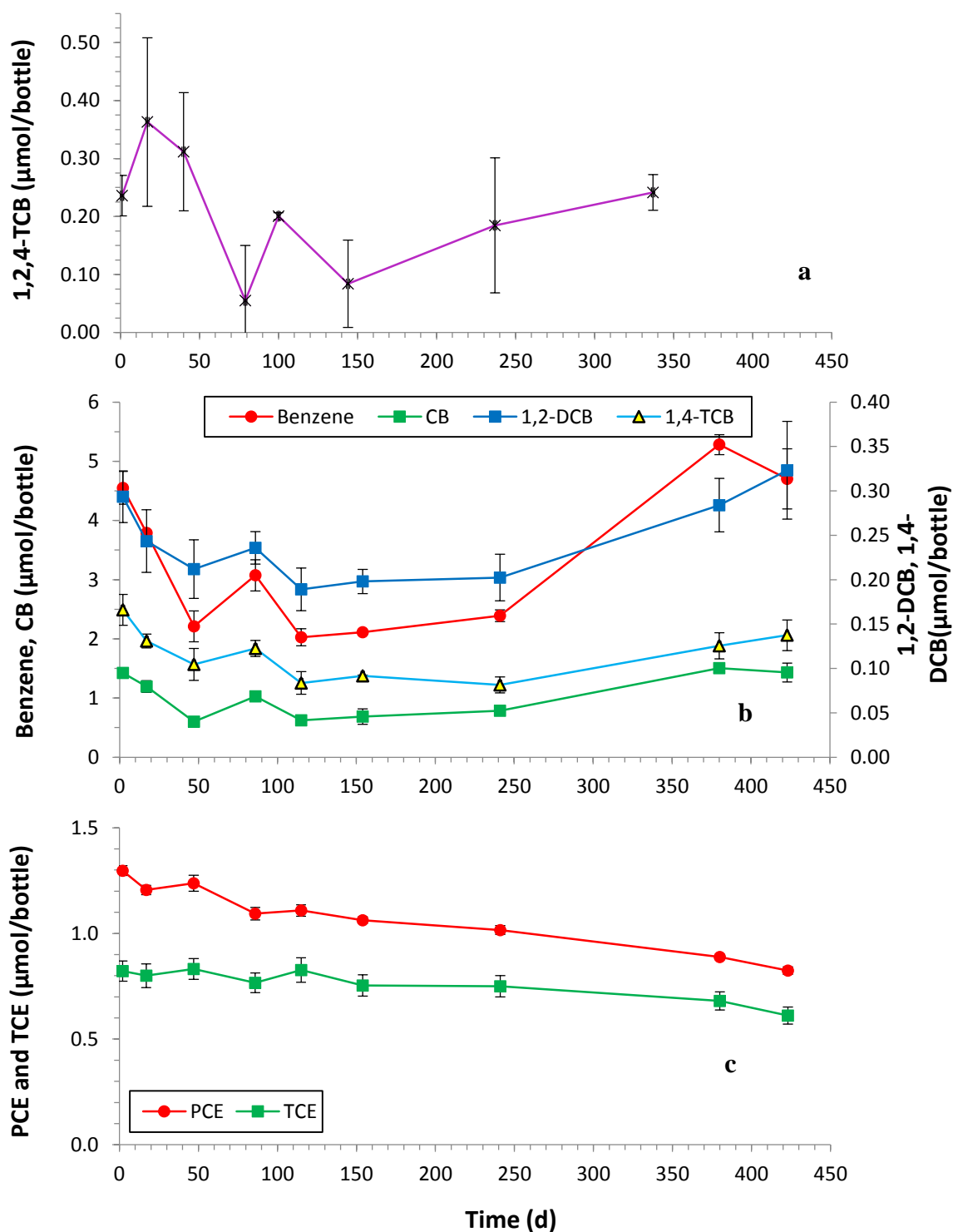


Figure 3.1. a) Water controls for 1,2,4-TCB; b) 1,2-DCB, 1,4-DCB, CB and benzene; and c) PCE and TCE. Error bars represent standard deviation.

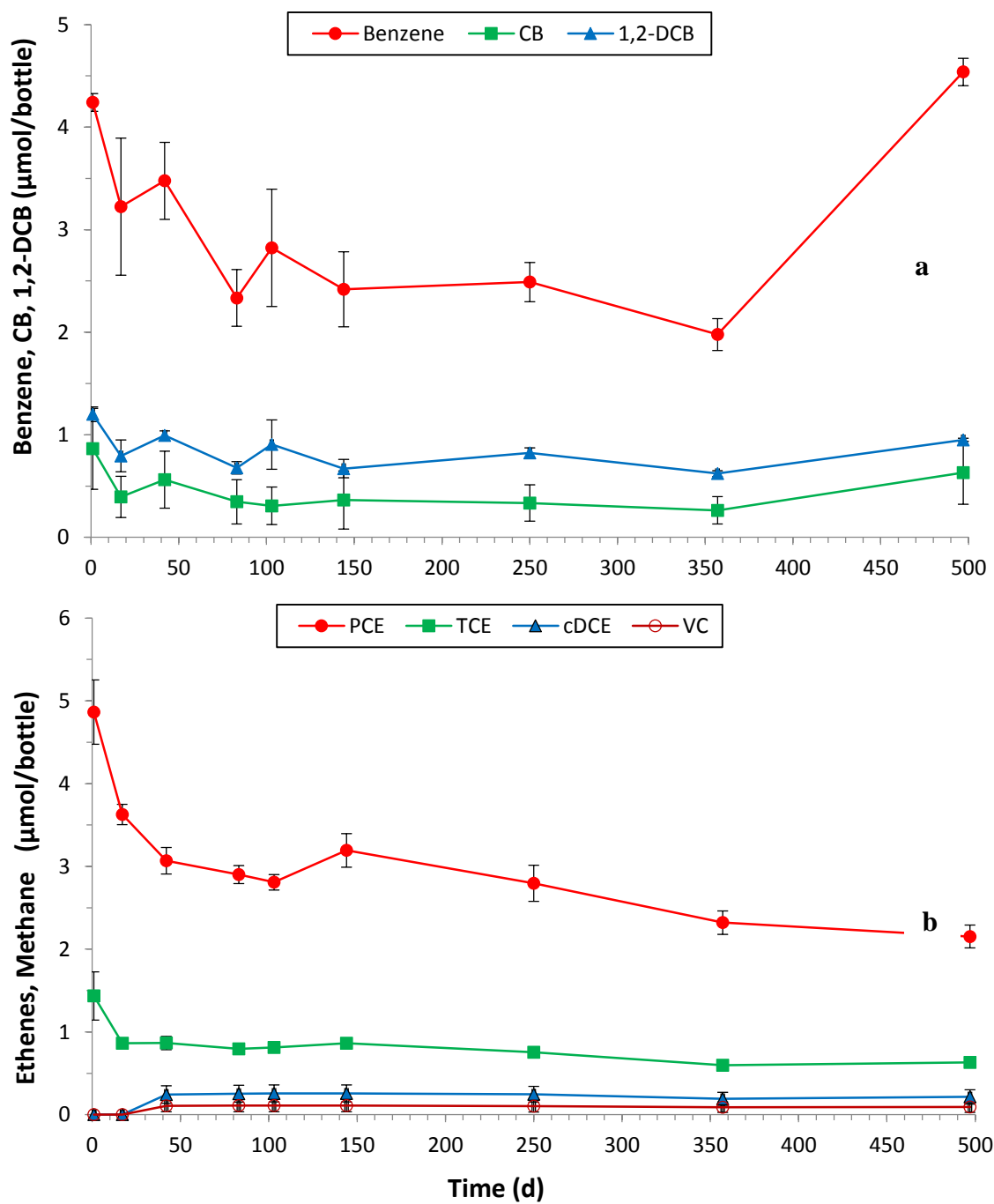


Figure 3.2. Results for the triplicate WWTP autoclaved control microcosms; error bars represent standard deviations; a) aromatic compounds at high concentrations; and b) chlorinated ethenes.

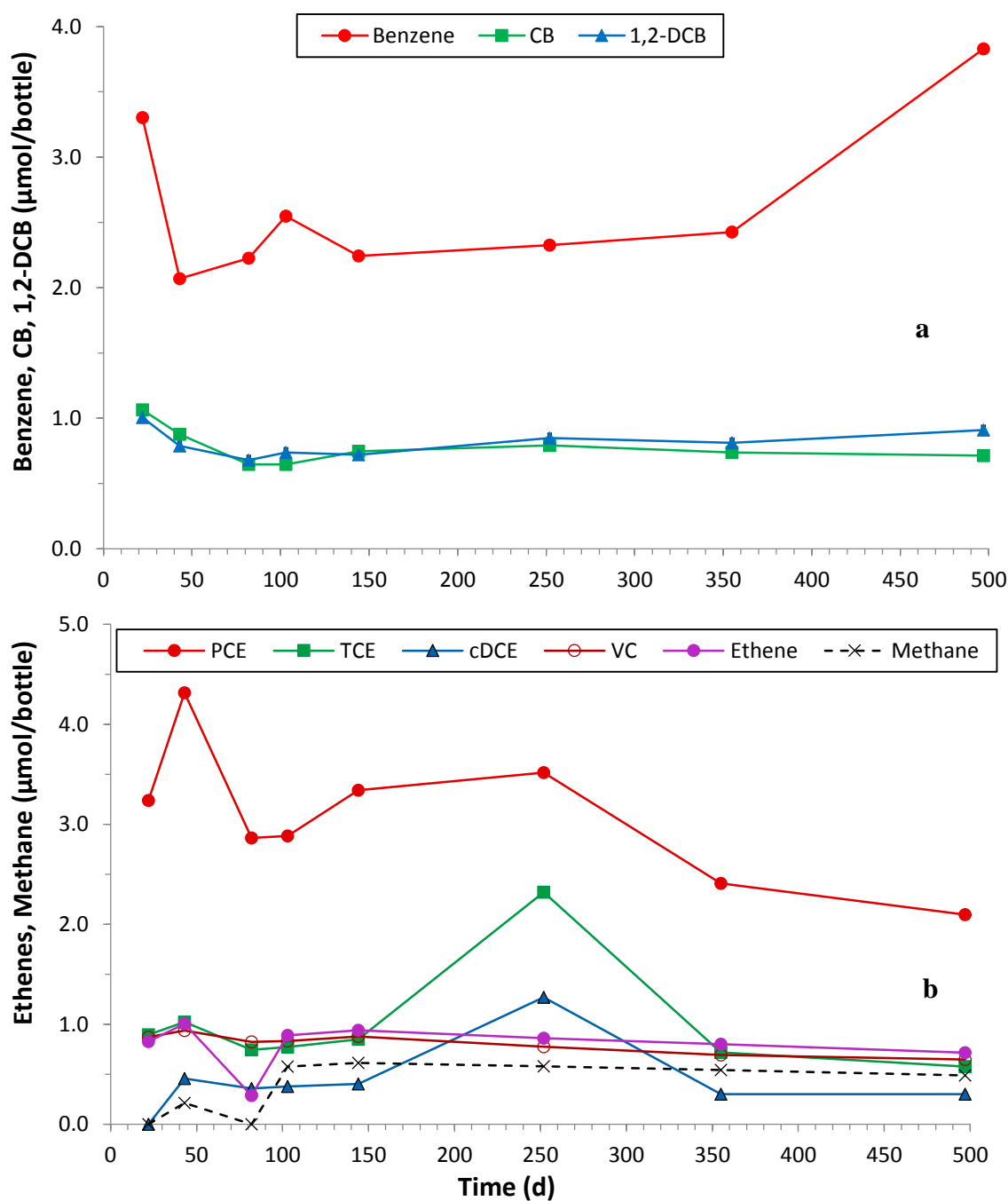


Figure 3.3. Results for the triplicate WWTP unamended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene and methane.

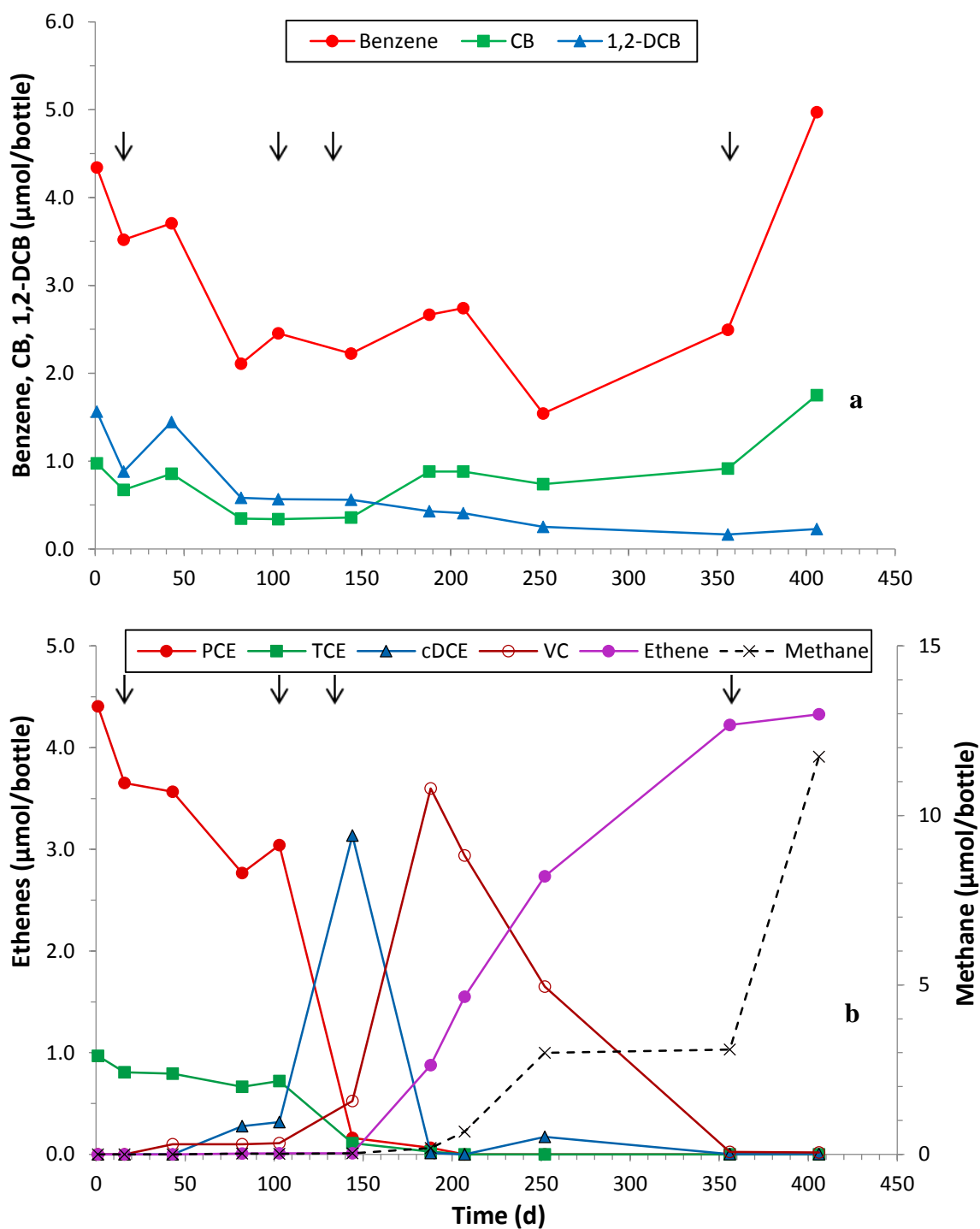


Figure 3.4. Results for the triplicate WWTP anaerobic lactate-amended microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate.

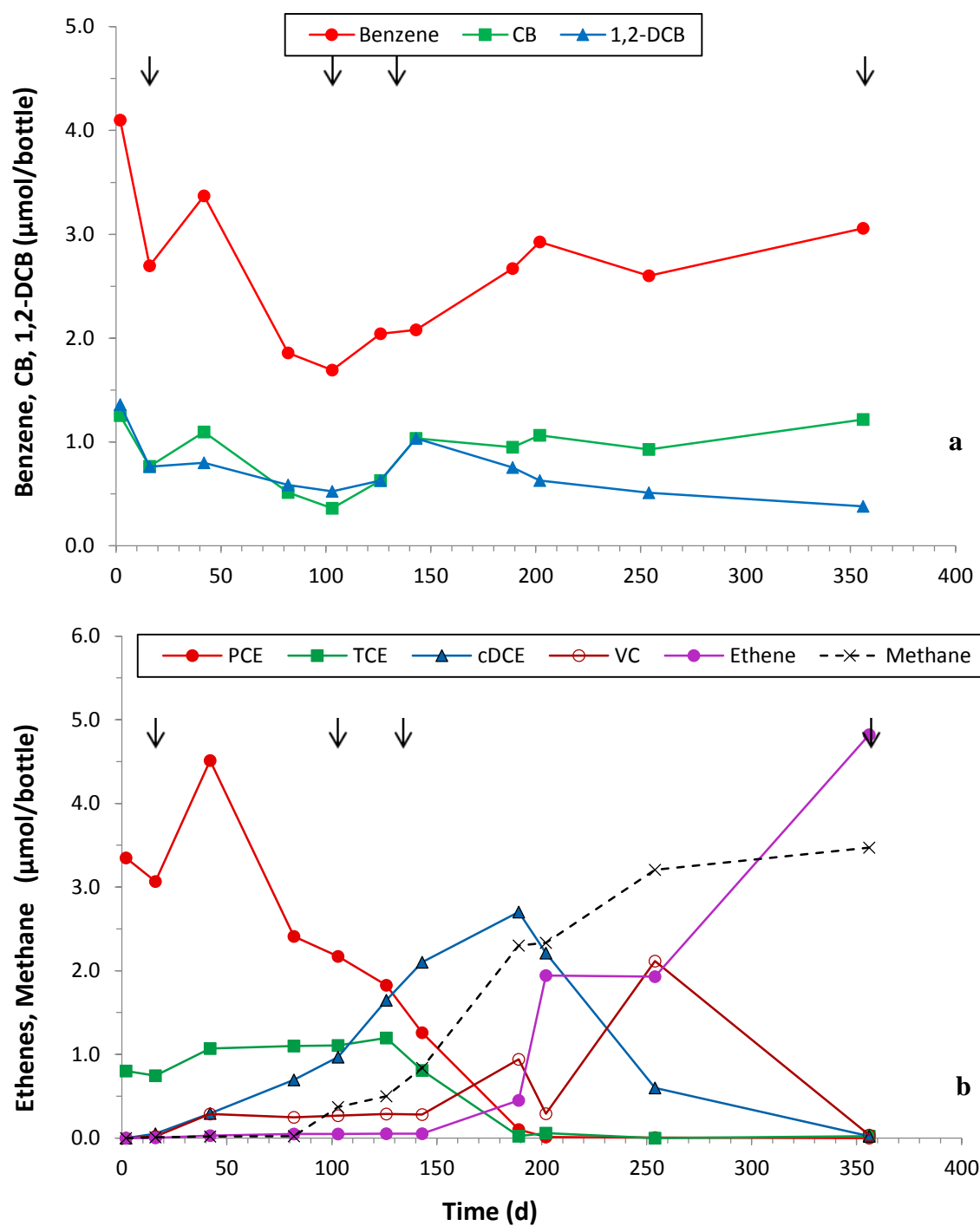


Figure 3.5. Results for the triplicate WWTP EVO-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of EVO.

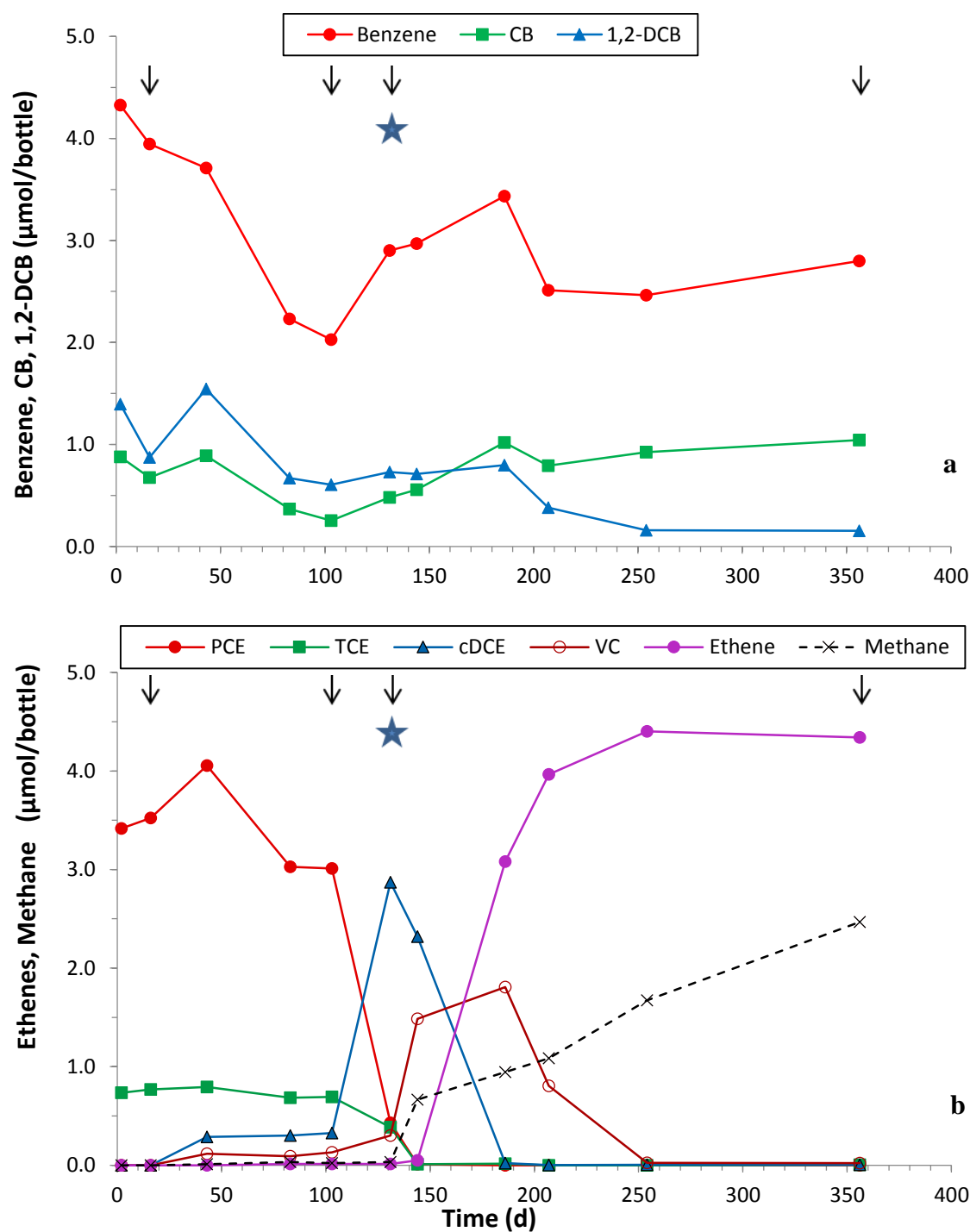


Figure 3.6. Results for the triplicate WWTP bioaugmented anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate and stars indicate addition of the KB-1 bioaugmentation culture.

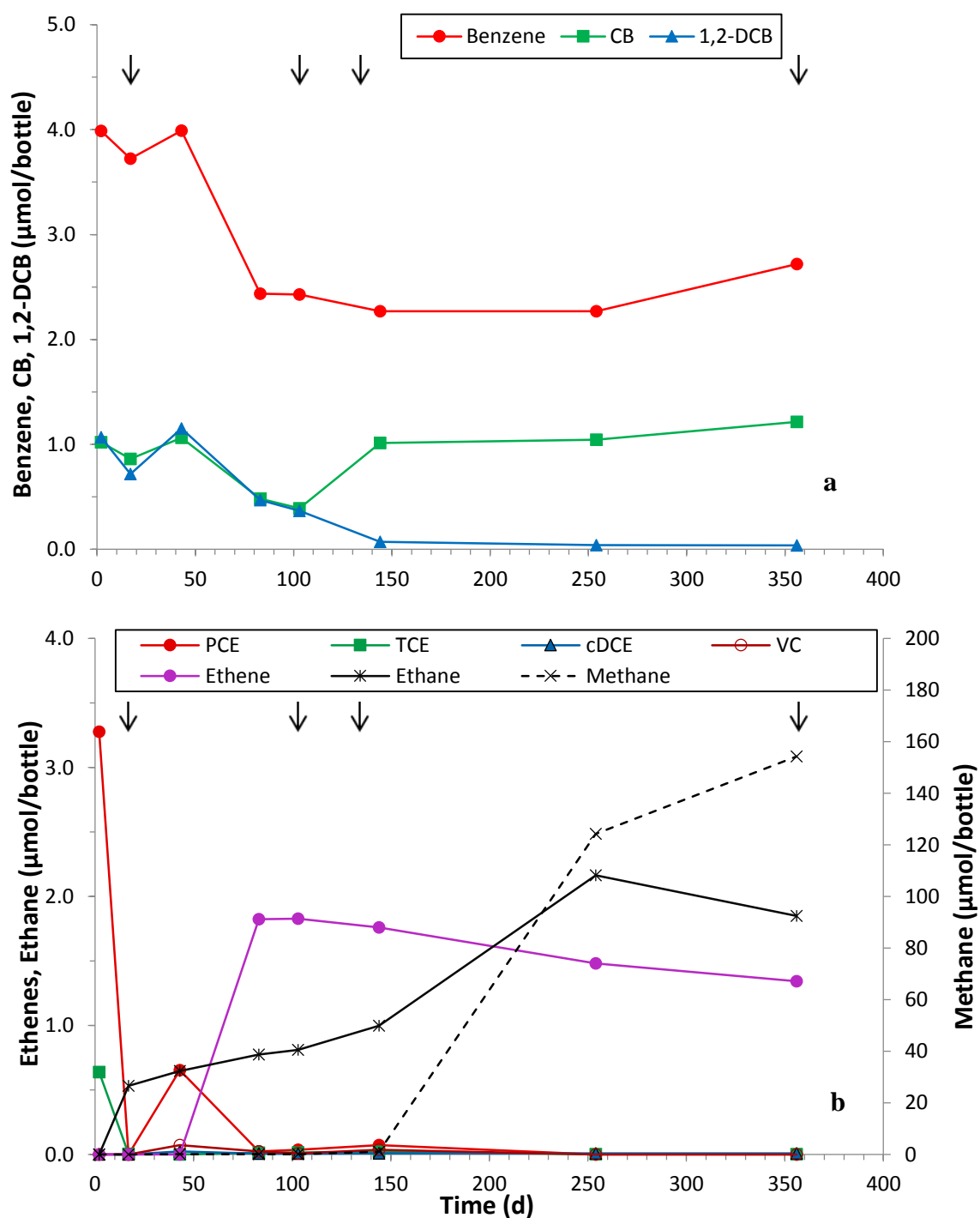


Figure 3.7. Results for the triplicate WWTP ZVI and lactate-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate.

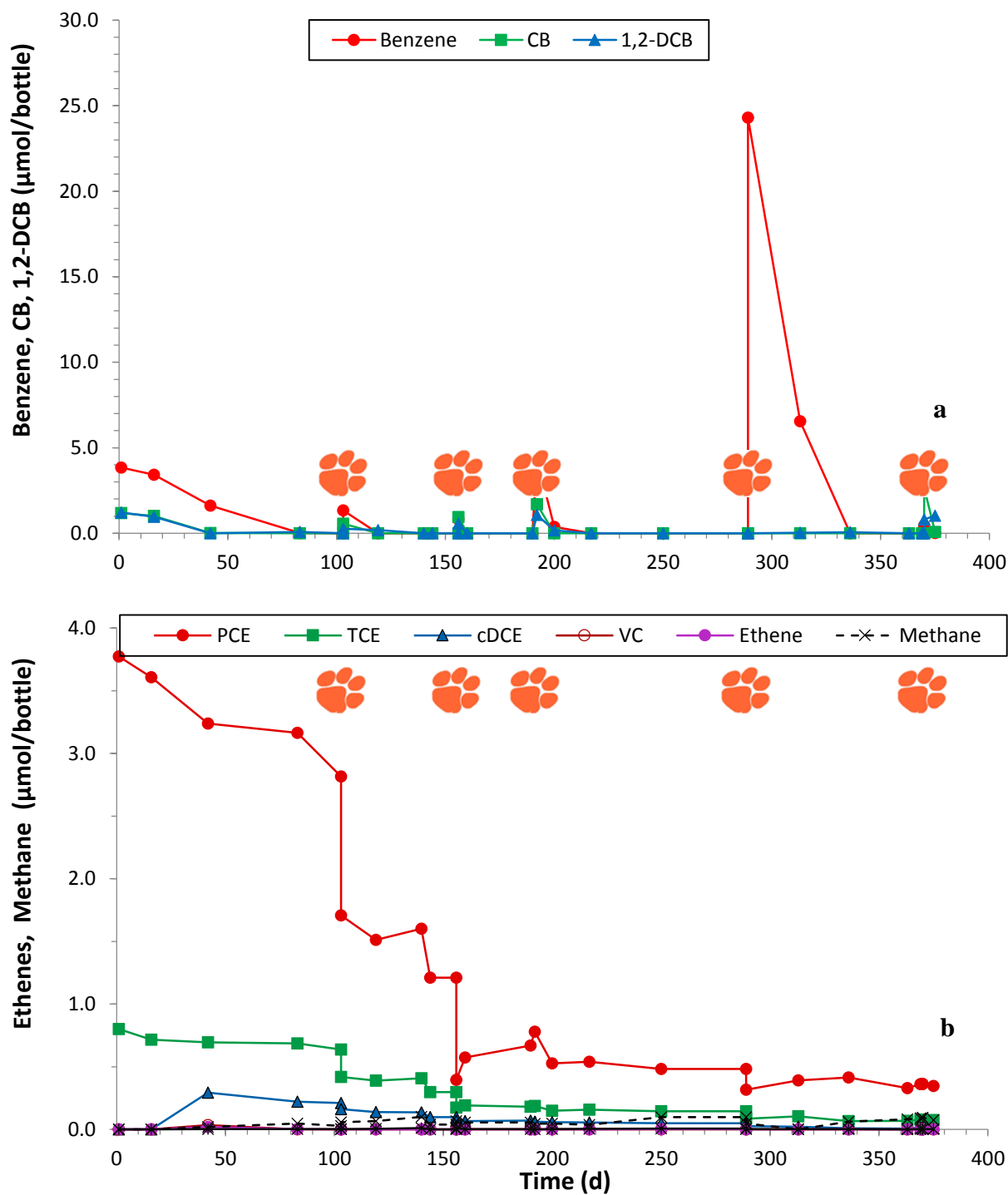


Figure 3.8. Results for the triplicate WWTP unamended aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Tiger paws indicate re-addition of chlorinated benzenes and benzene.

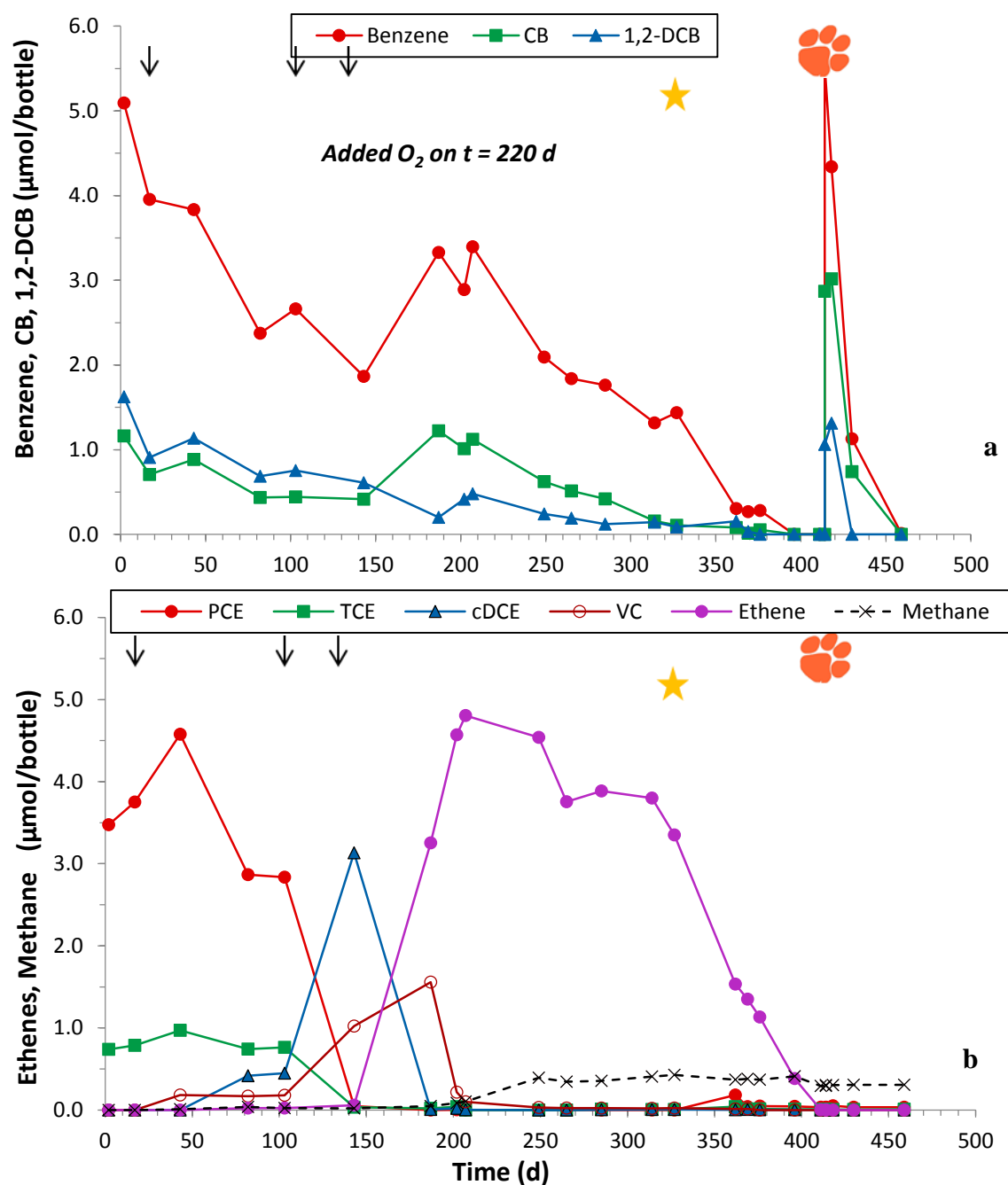


Figure 3.9. Results for the triplicate WWTP lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate; stars indicate addition soil from WWTP-AER microcosms; and the tiger paws indicate re-addition of chlorinated benzenes and benzene.

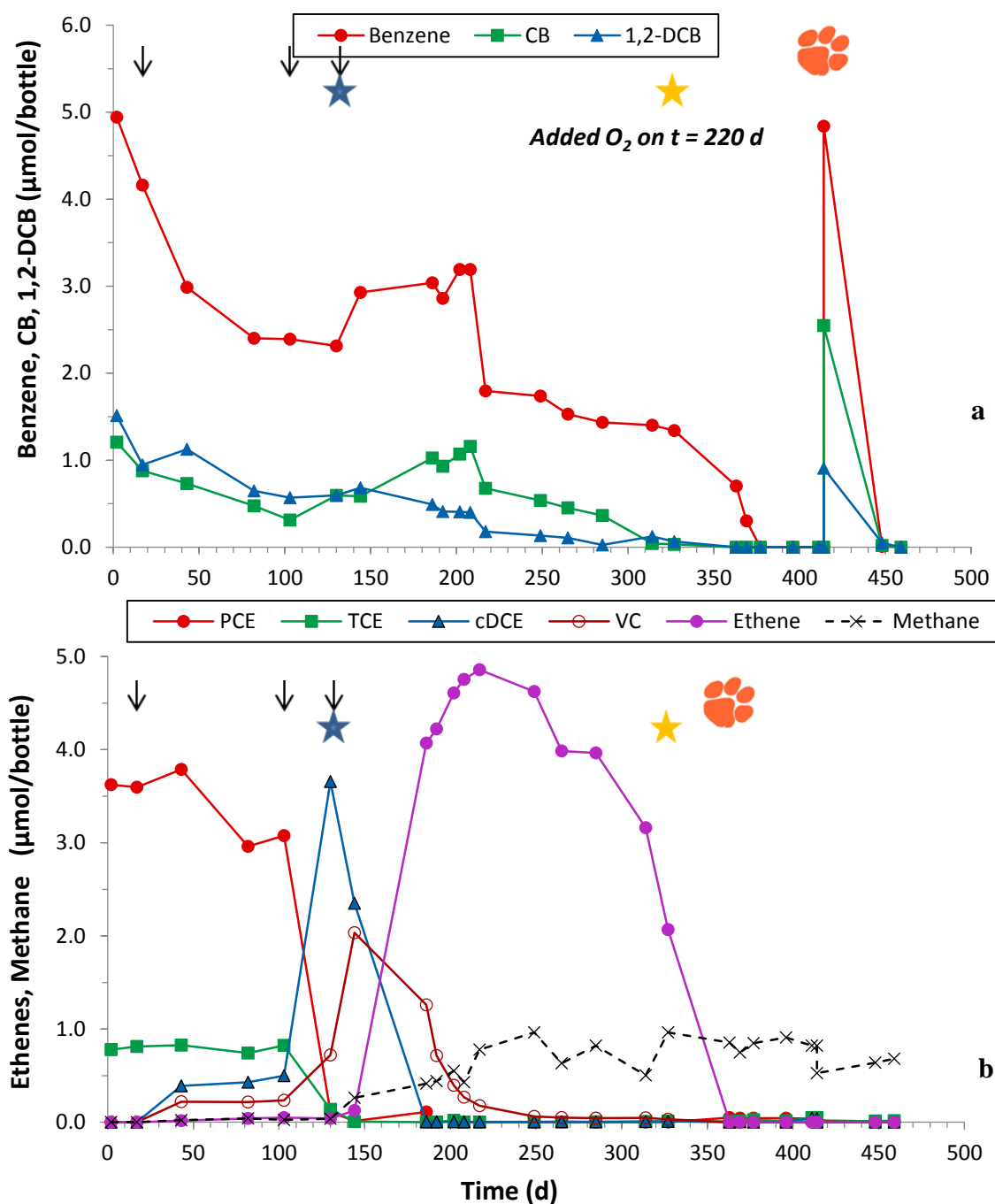


Figure 3.10. Results for the triplicate WWTP bioaugmented anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture and yellow stars indicate addition of soil from the WWTP-AER microcosms.

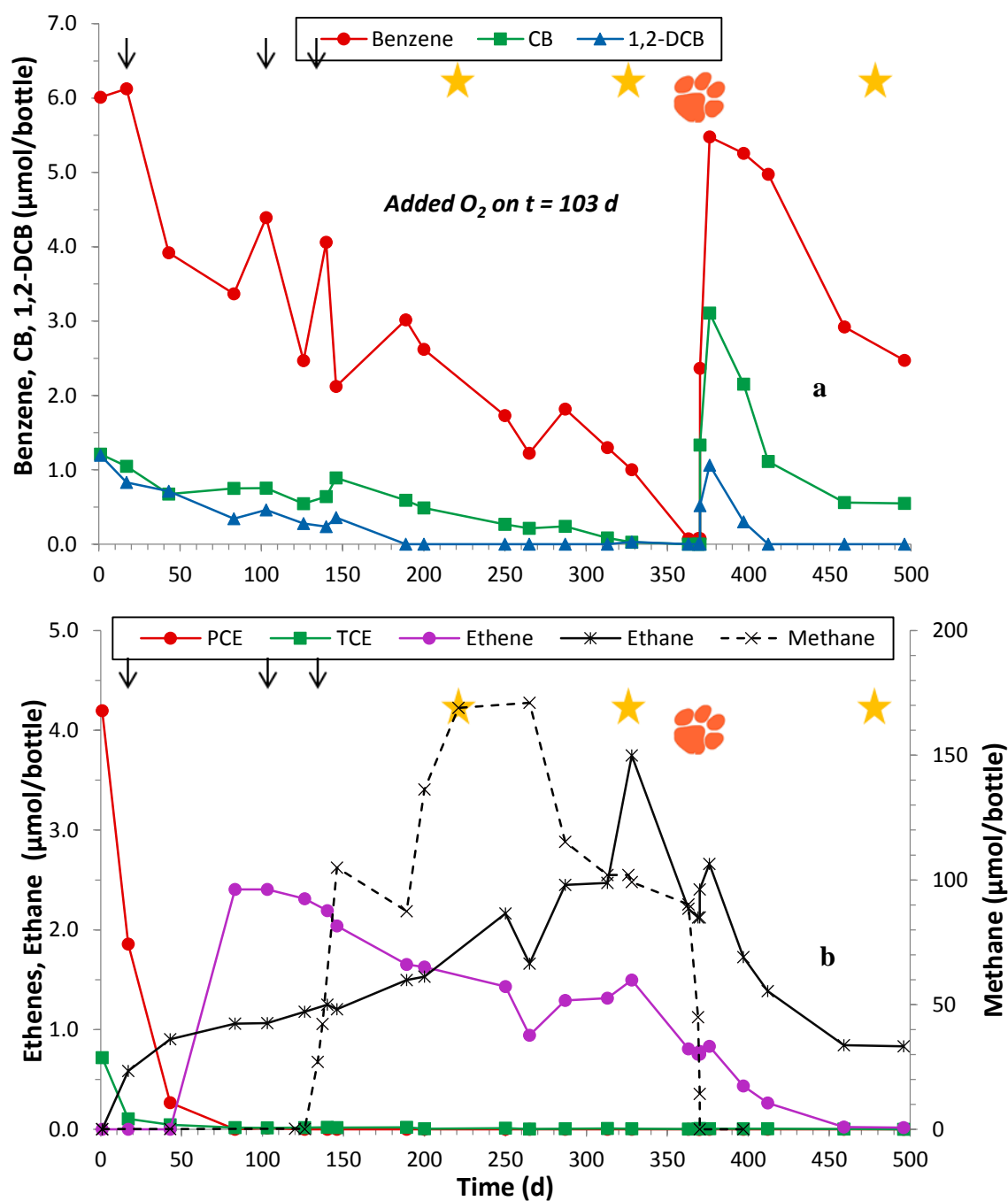


Figure 3.11. Results for the triplicate WWTP ZVI and lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate; stars indicate addition of soil from WWTP-AER microcosm #2, and tiger paws indicate re-addition of chlorinated benzenes and benzene.

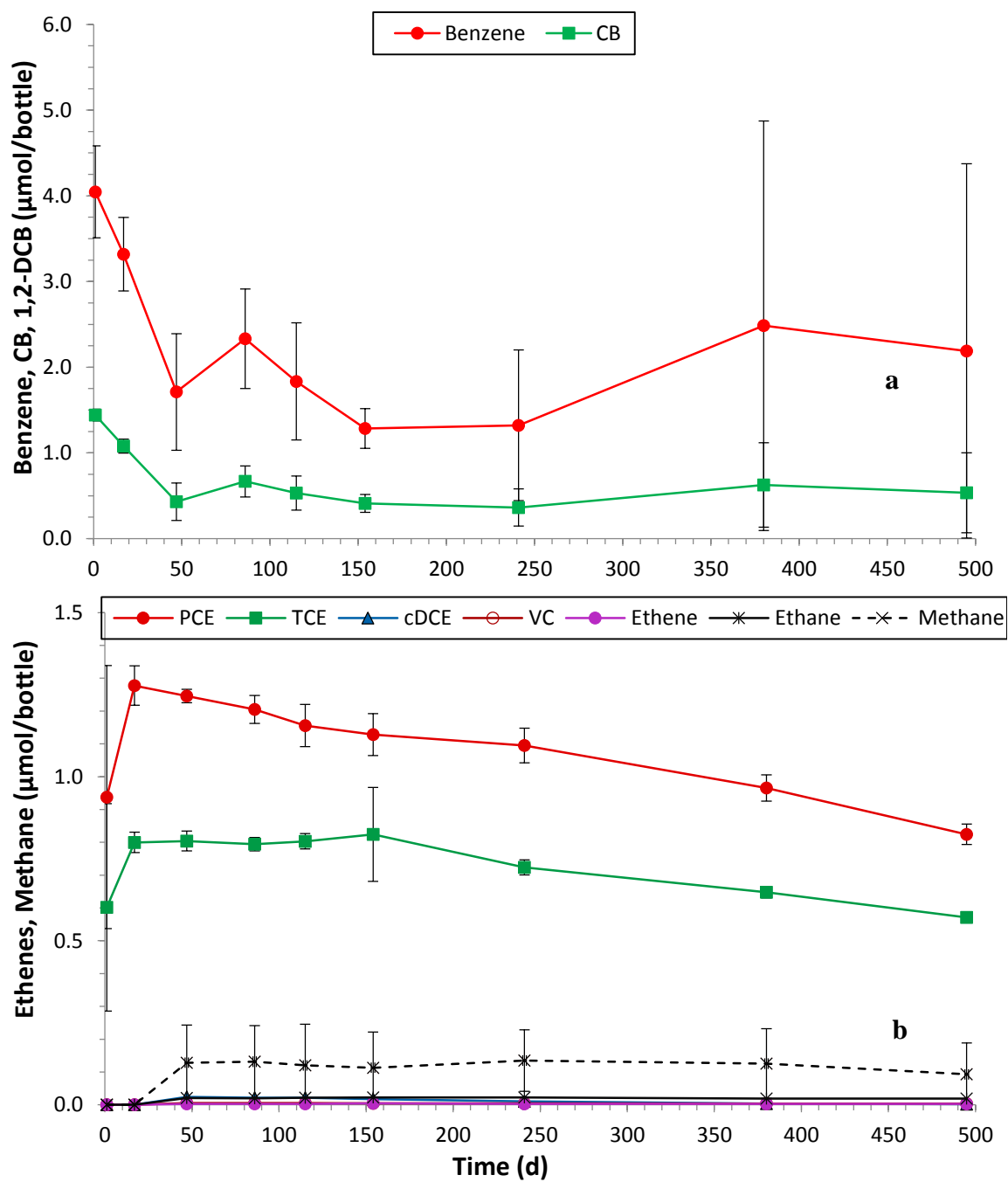


Figure 3.12. Results for the triplicate ORP autoclaved control microcosms; error bars represent standard deviations; a) CB and benzene; and b) PCE and TCE.

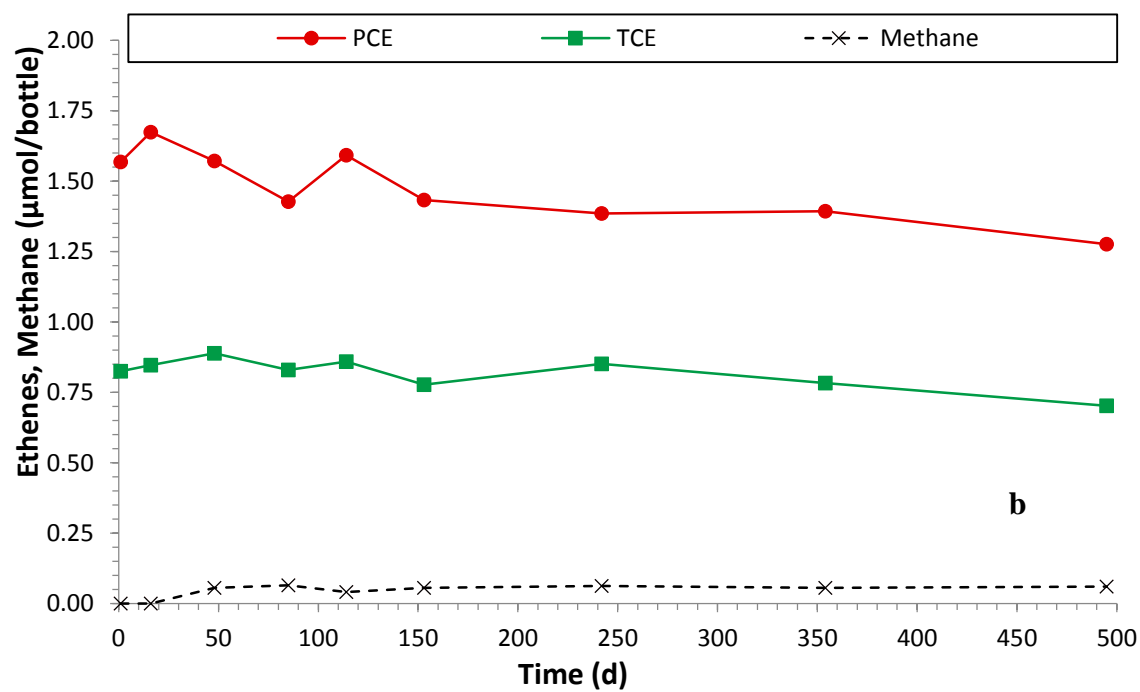
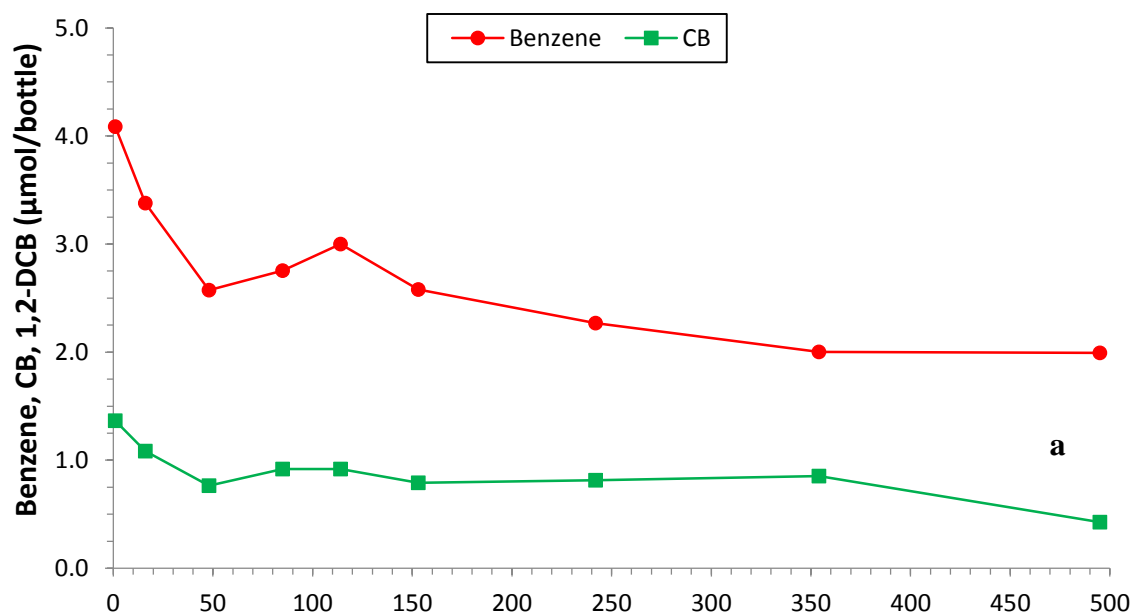


Figure 3.13. Results for the triplicate ORP unamended anaerobic microcosms; a) CB and benzene; and b) PCE, TCE and methane.

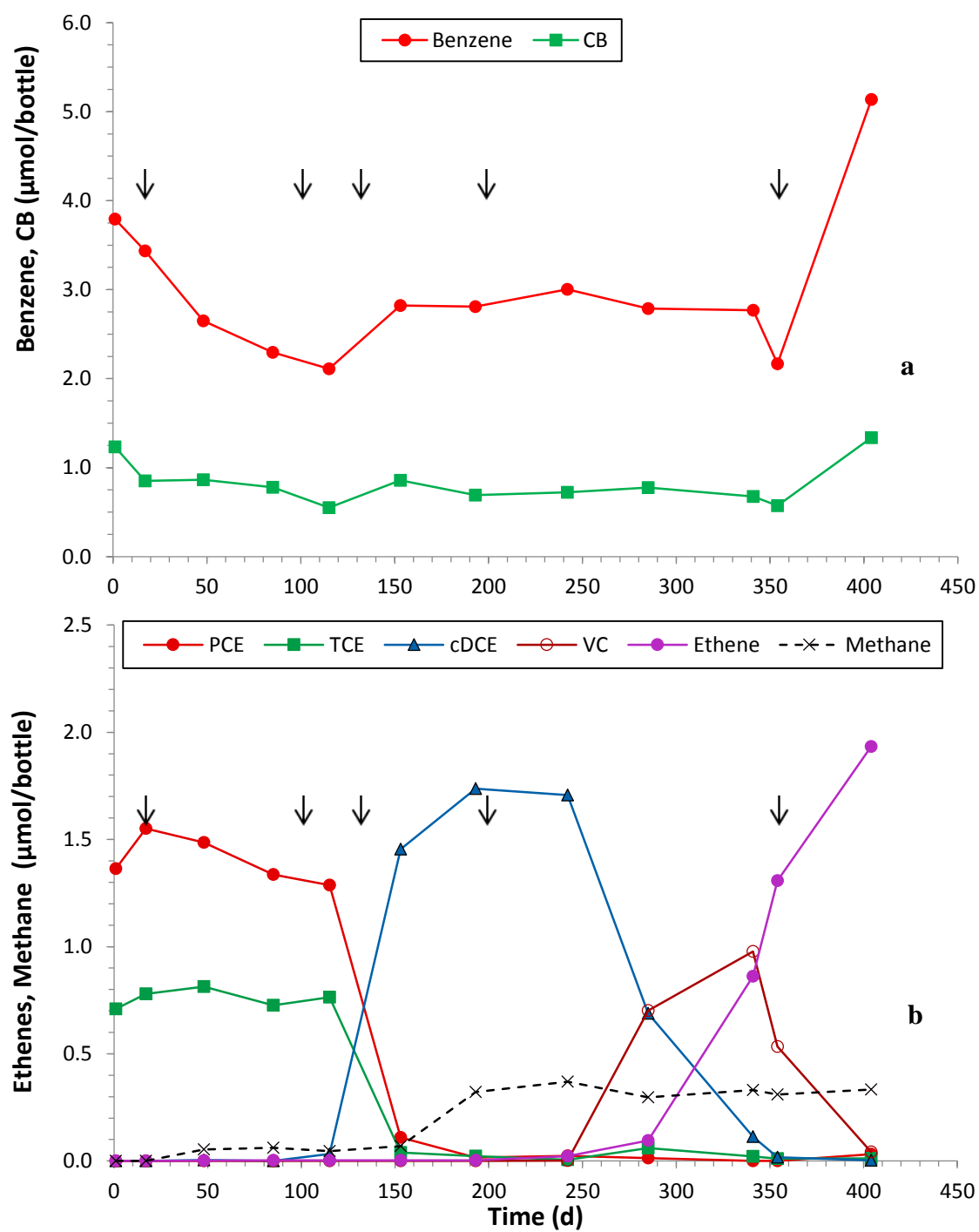


Figure 3.14. Results for the ORP anaerobic lactate-amended microcosm #1; a) CB and benzene; and b) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate.

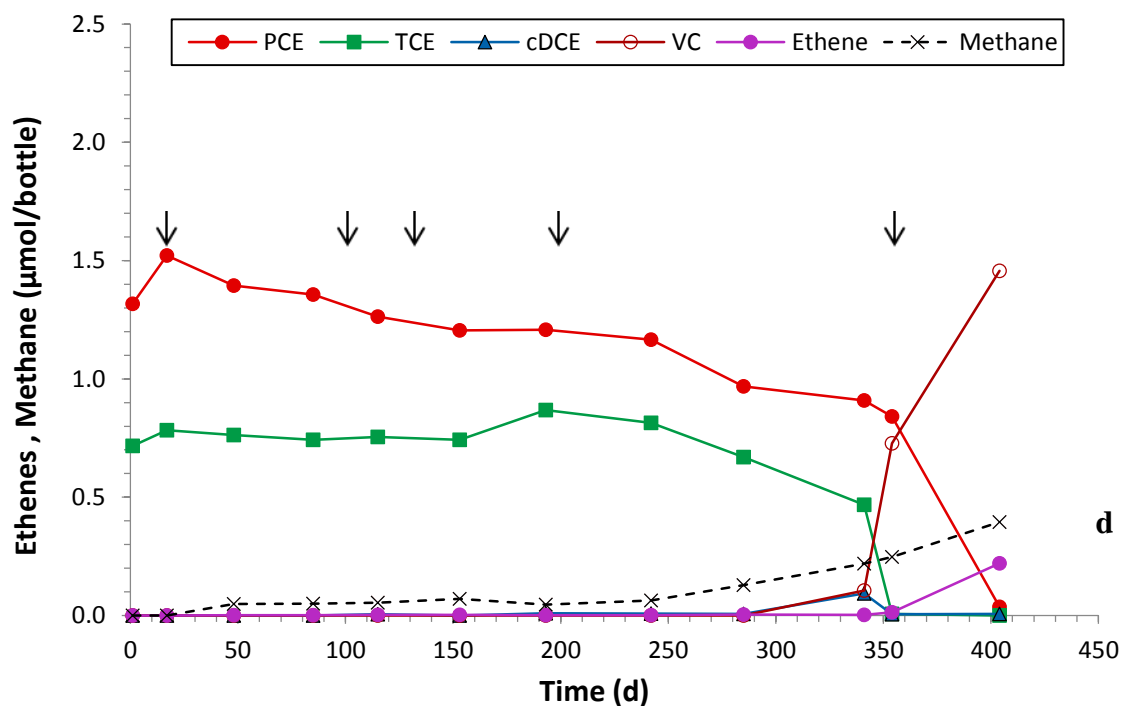
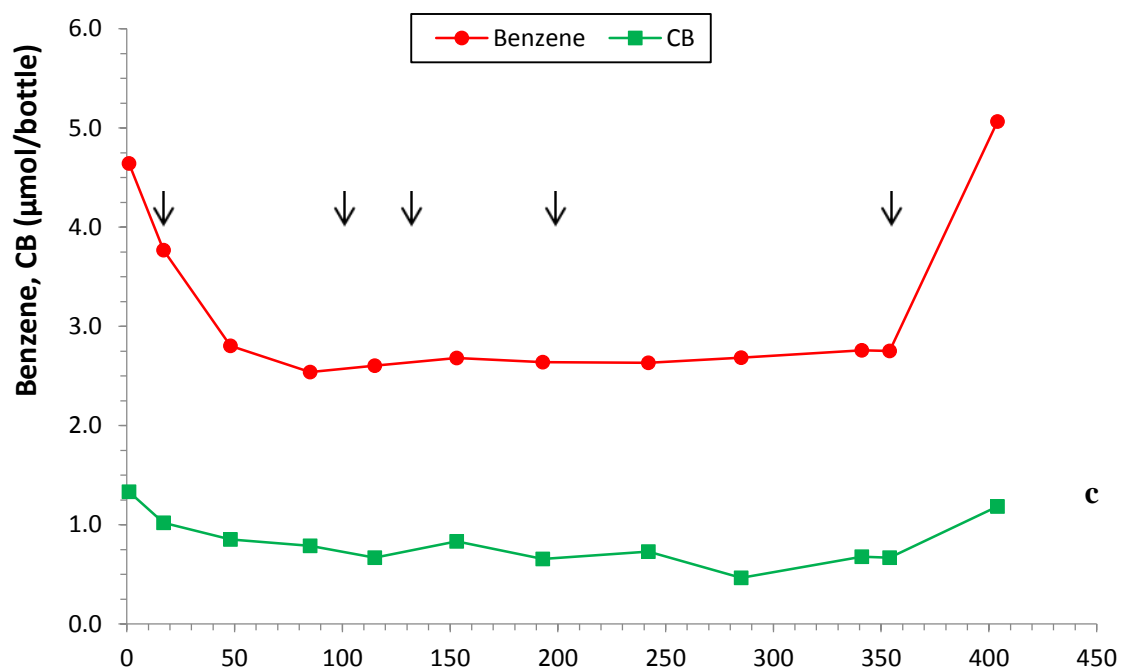


Figure 3.14 (continued). Results for the ORP anaerobic lactate-amended microcosm #2; c) CB and benzene; and d) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate.

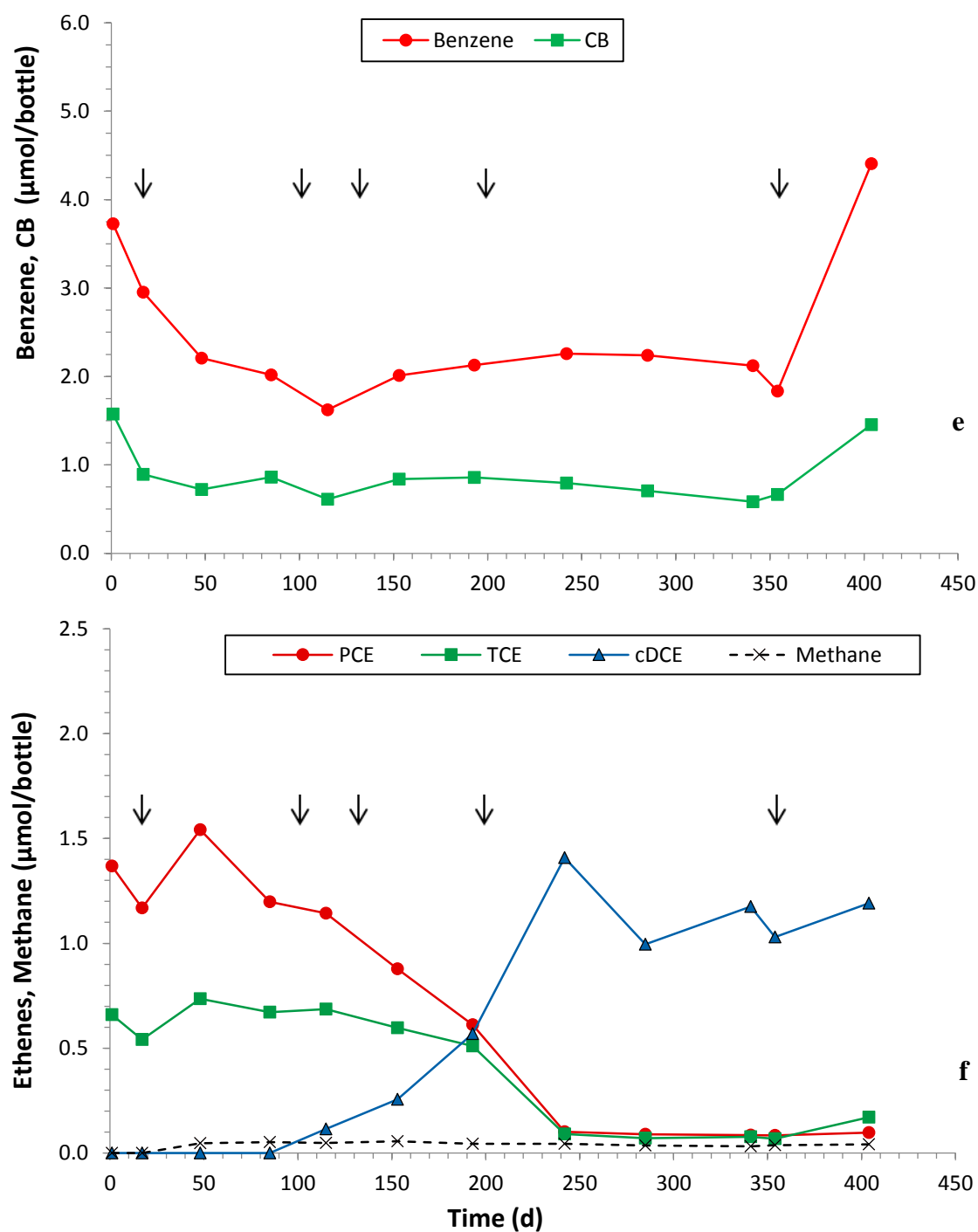


Figure 3.14 (continued). Results for the ORP anaerobic lactate-amended microcosm #3; e) CB and benzene; and f) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate.

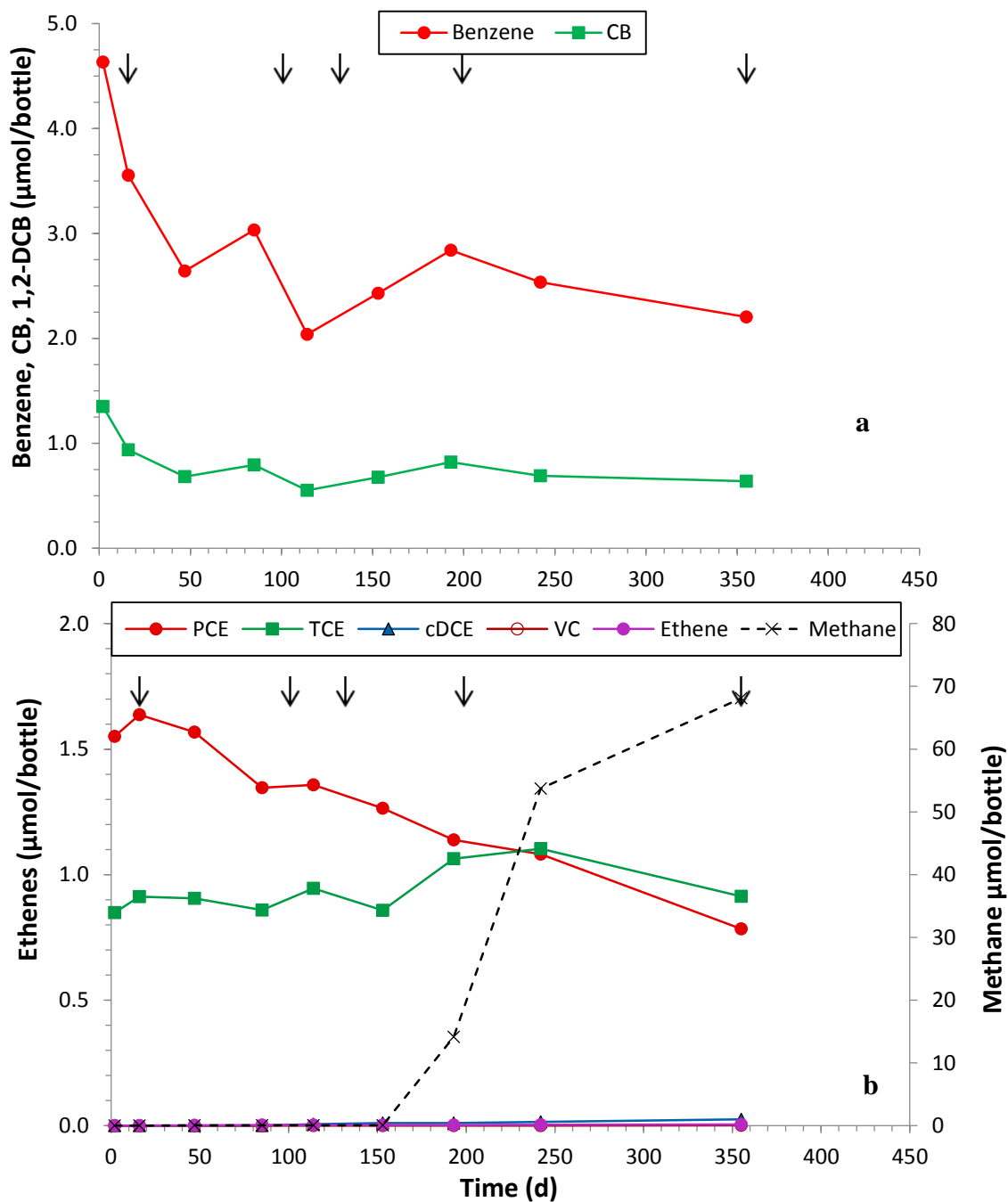


Figure 3.15. Results for the triplicate ORP EVO-amended anaerobic microcosms; a) CB and benzene; and b) chlorinated ethenes and methane. Arrows indicate addition of EVO.

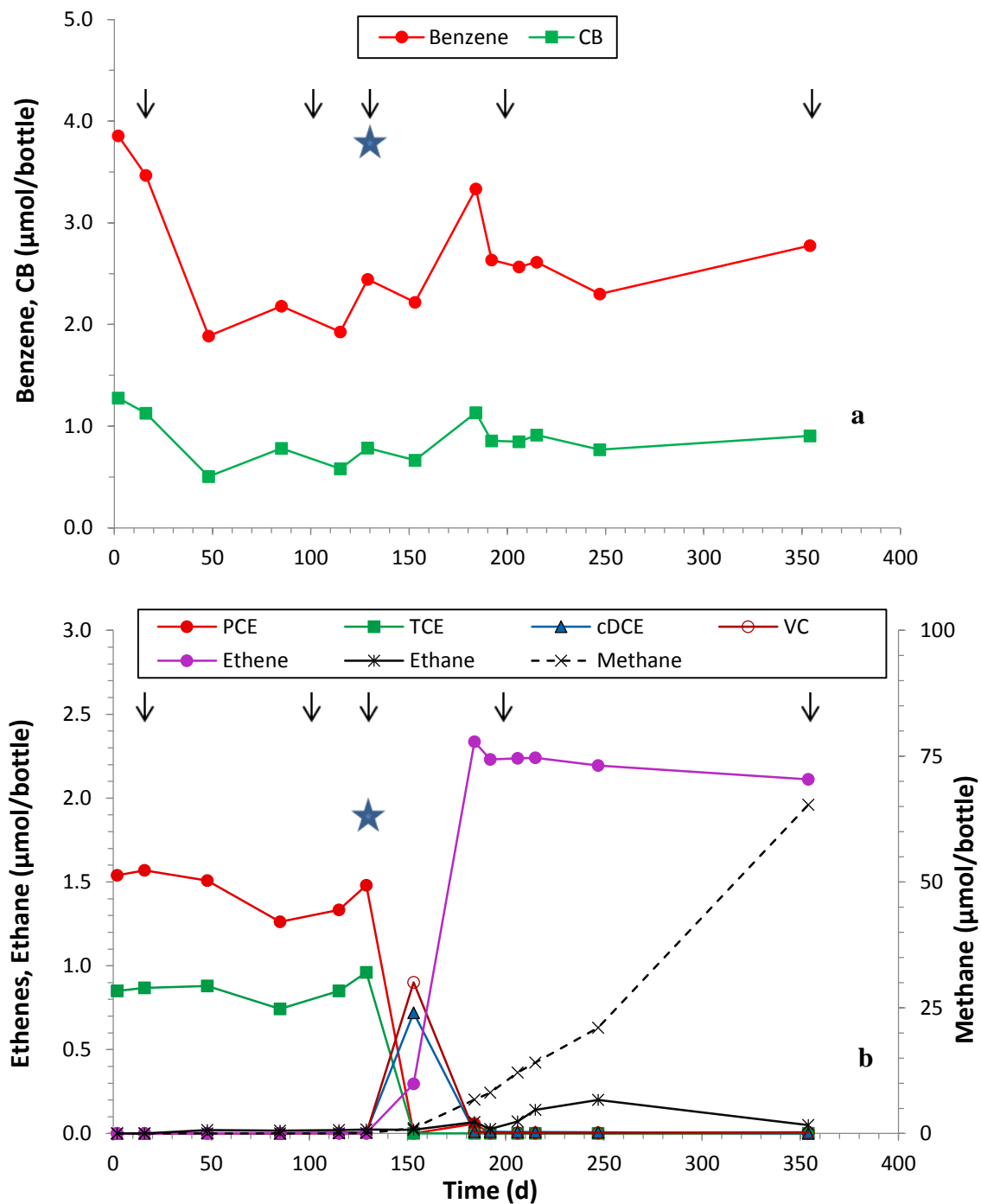


Figure 3.16. Results for the triplicate ORP bioaugmented anaerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate and stars indicate addition of the KB-1 bioaugmentation culture.

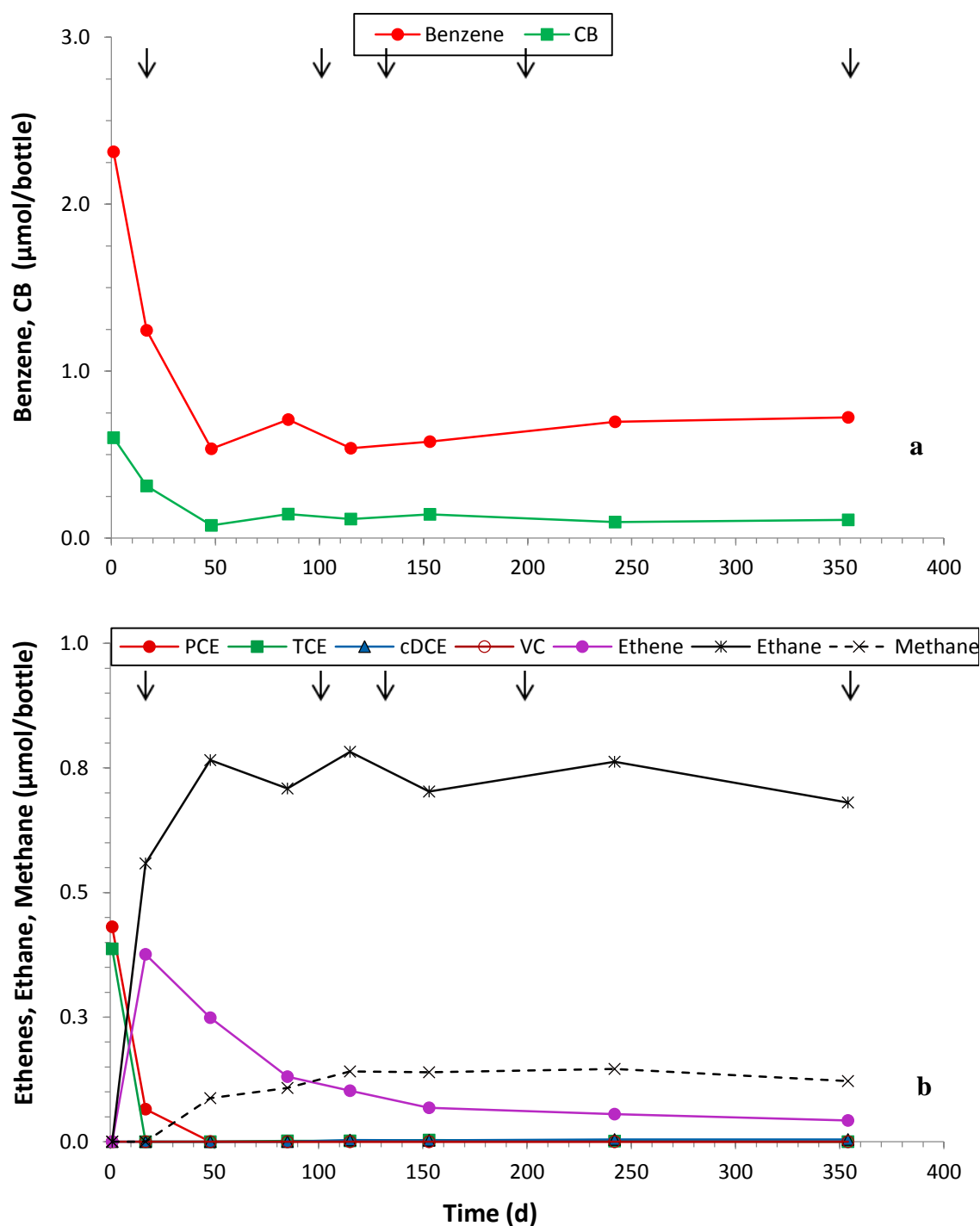


Figure 3.17. Results for the triplicate ORP ZVI and lactate-amended anaerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate.

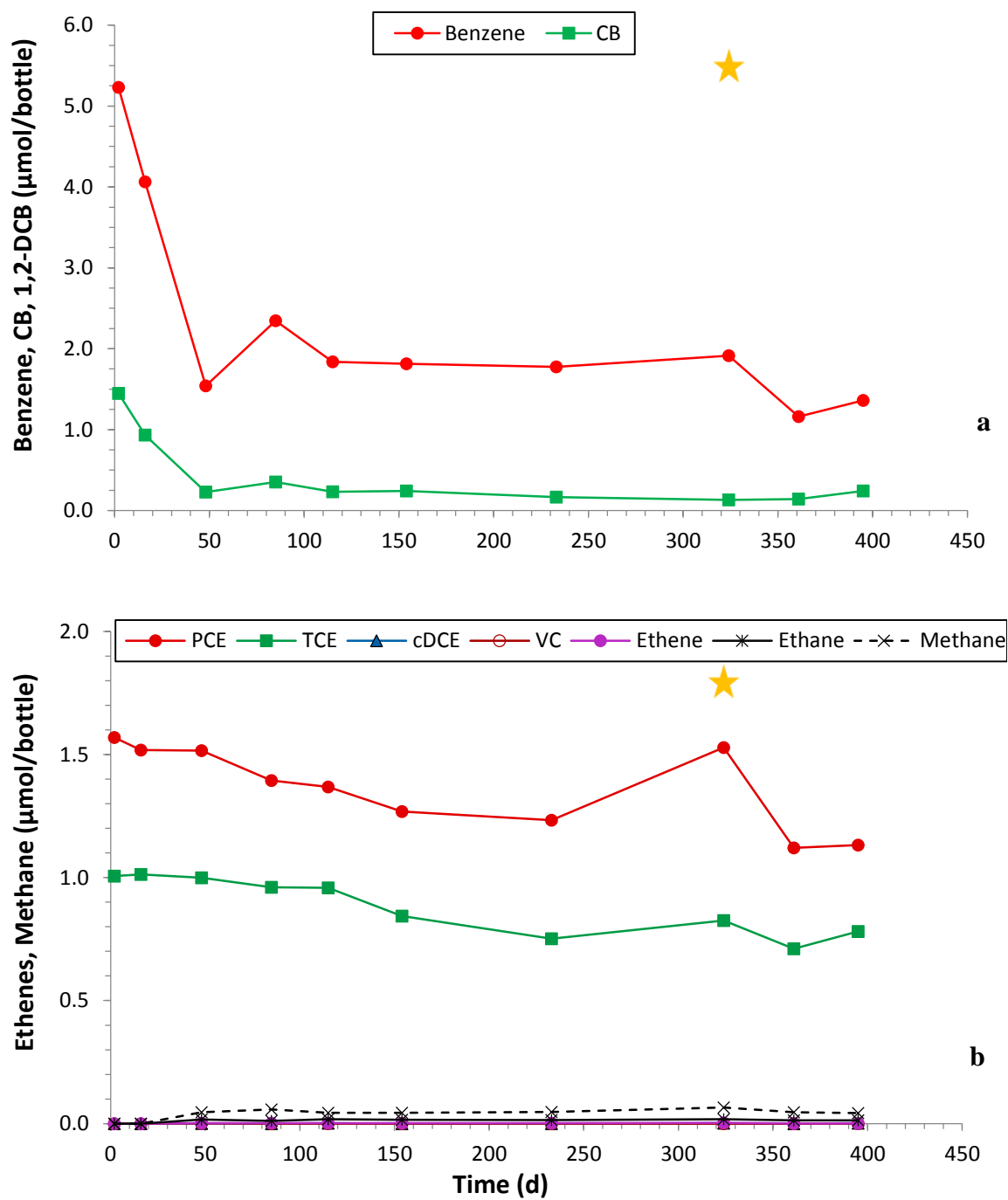


Figure 3.18. Results for the triplicate ORP unamended aerobic microcosms; a) CB and benzene; and b) PCE, TCE, and methane. Yellow stars indicate addition of soil and groundwater from the WWTP-AER microcosms.

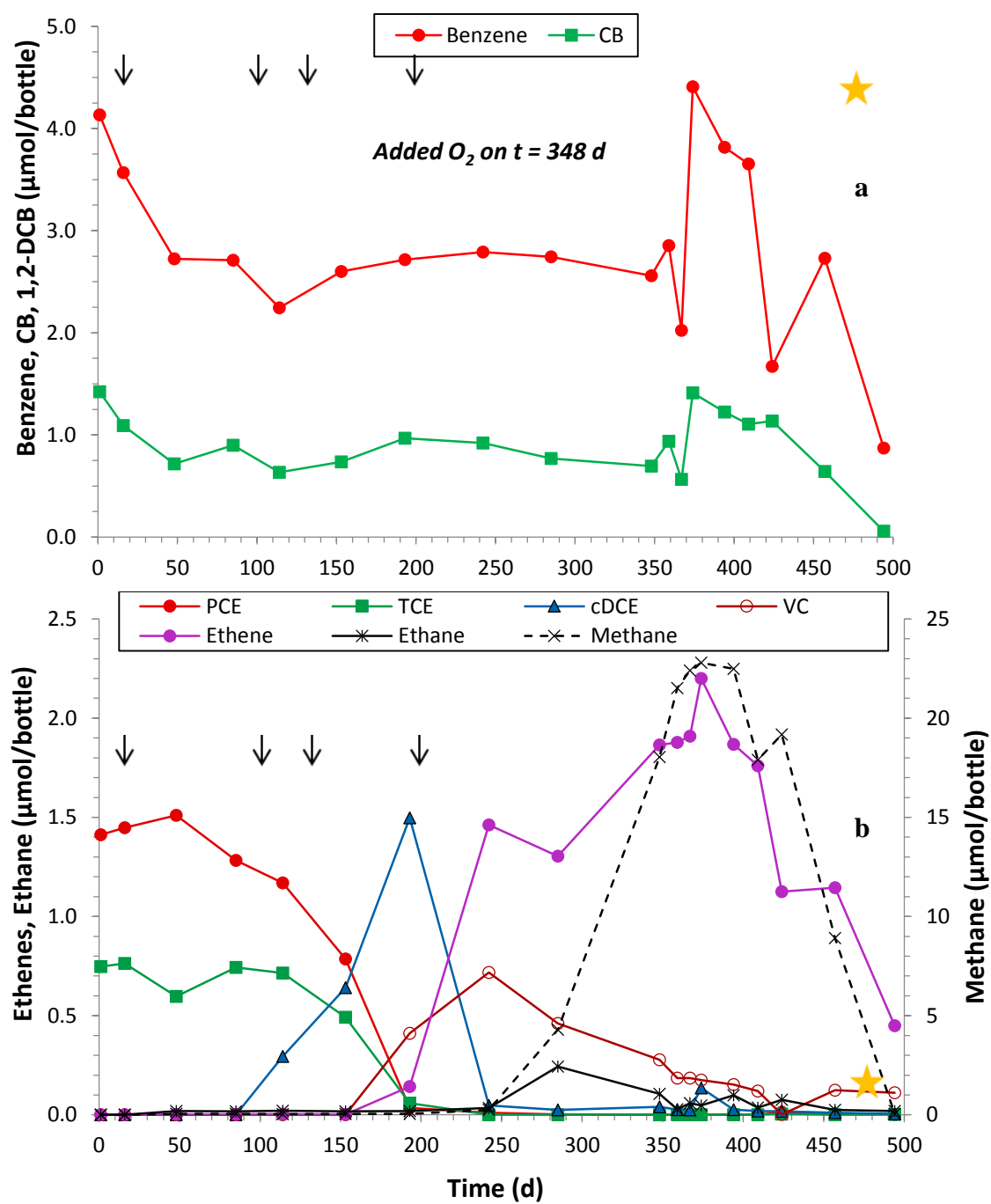


Figure 3.19. Results for the triplicate ORP lactate-amended anaerobic/aerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate.

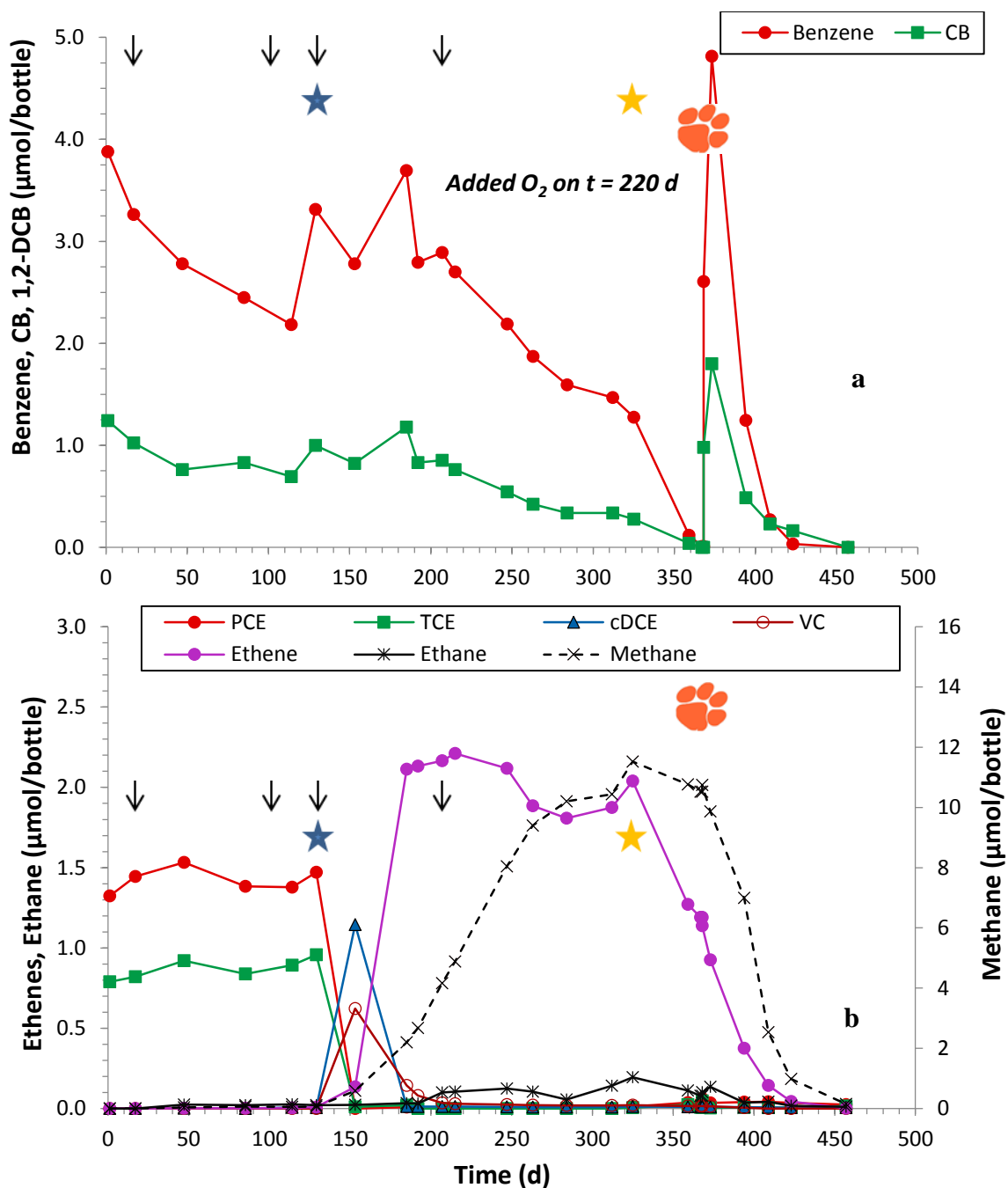


Figure 3.20. Results for the triplicate ORP bioaugmented anaerobic/aerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture; yellow stars indicate addition of soil and groundwater from the WWTP-AER microcosms; and tiger paws indicate re-addition of chlorinated benzenes and benzene.

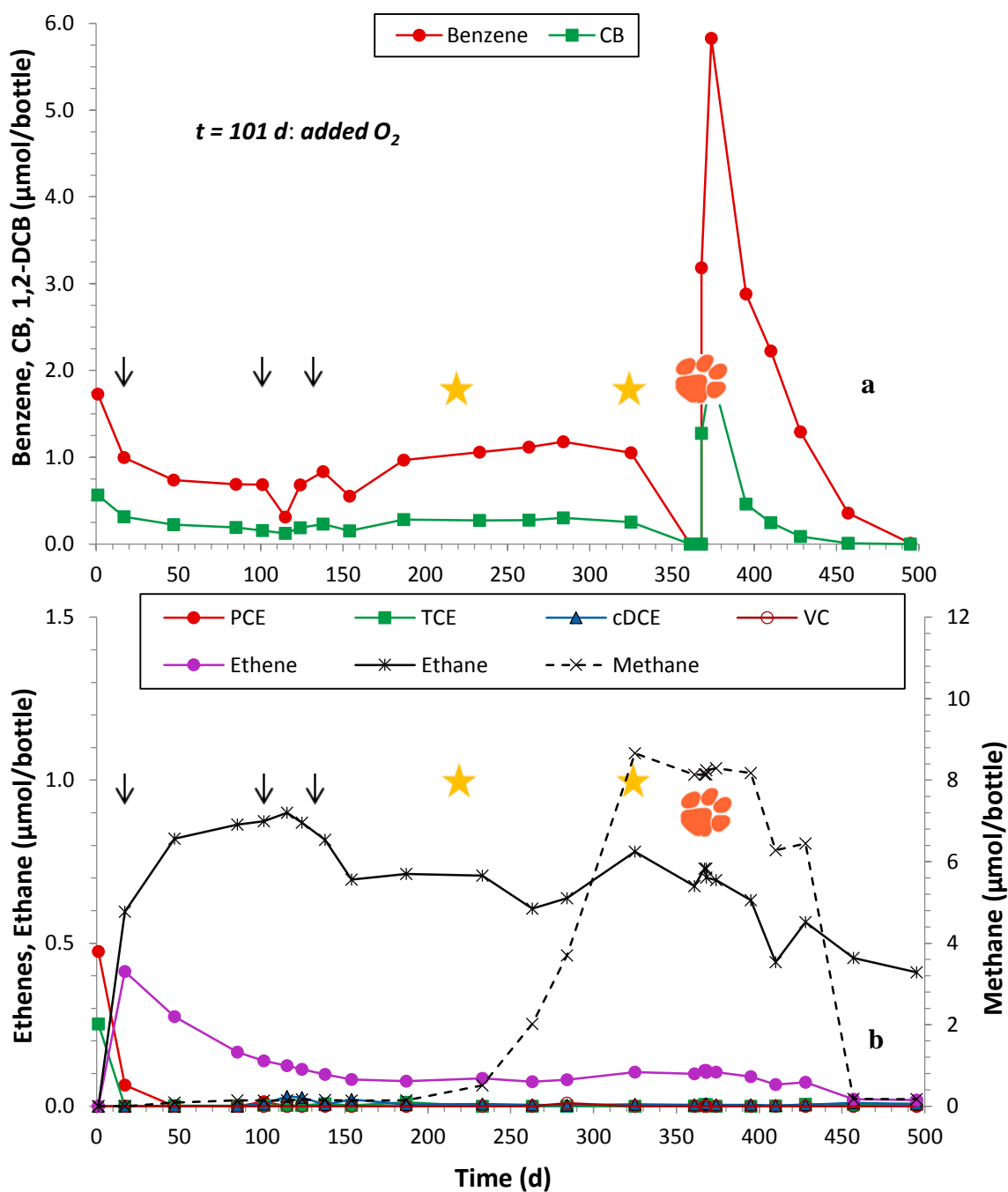


Figure 3.21. Results for the triplicate ORP ZVI and lactate-amended anaerobic/aerobic microcosms; a) CB and benzene; and b) chlorinated ethenes, ethene, ethane and methane. Arrows indicate an addition of lactate, stars indicate addition of soil from WWTP-AER series and tiger paws indicate addition of chlorinated benzenes and benzene.

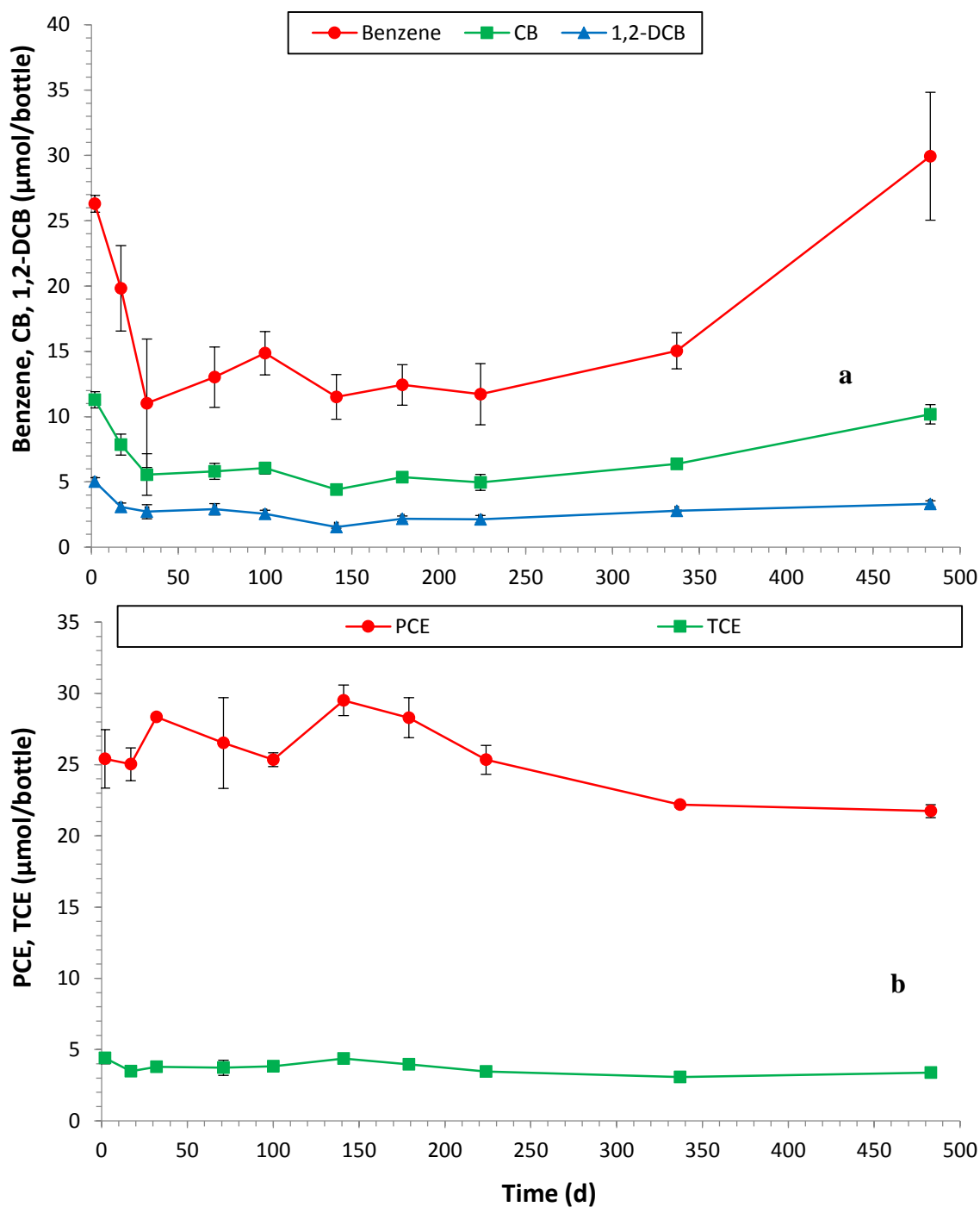


Figure 3.22. Results for the triplicate tank farm autoclaved control microcosms; error bars represent standard deviations; a) aromatic compounds at high concentrations; and b) PCE and TCE.

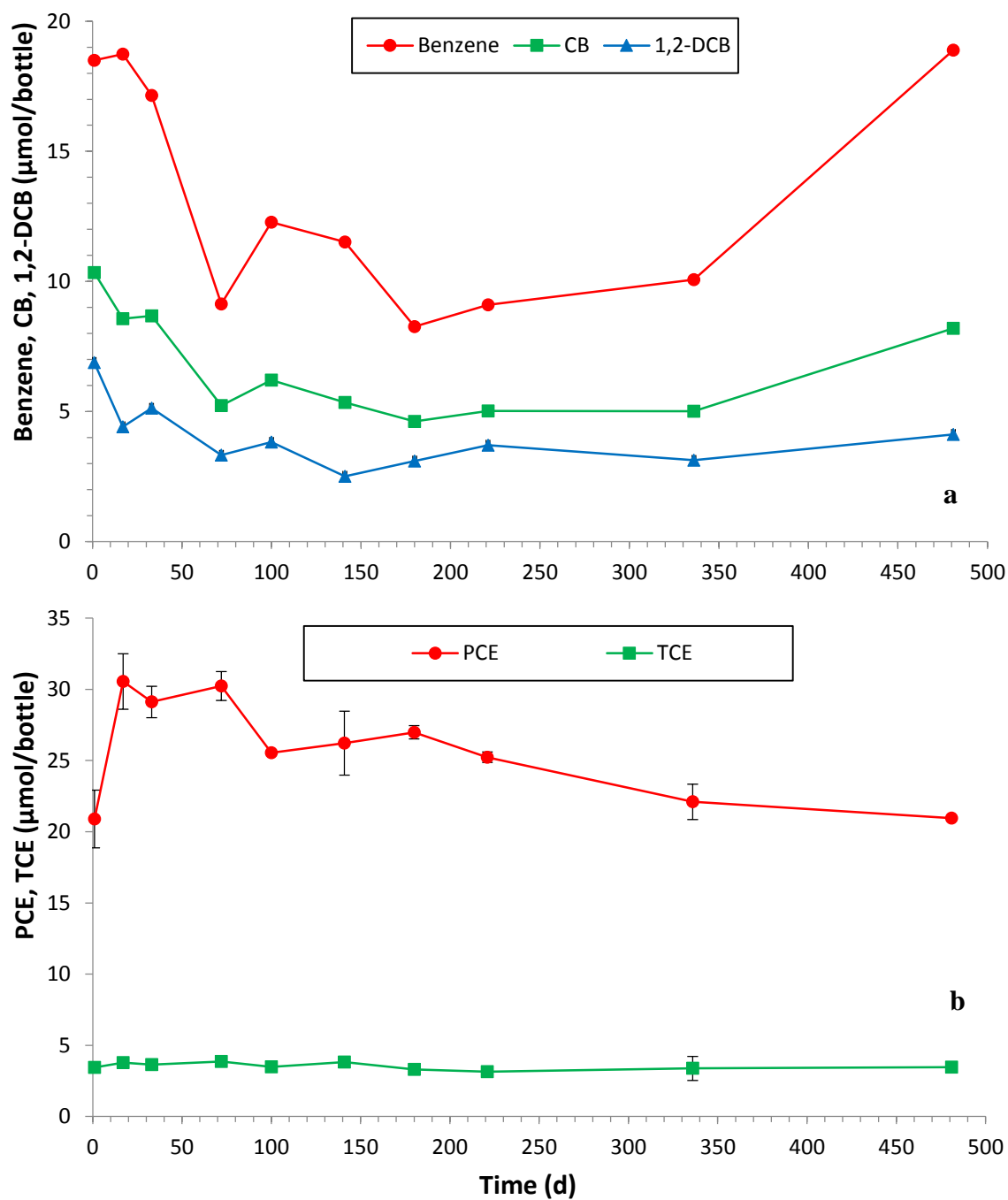


Figure 3.23. Results for the triplicate tank farm unamended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE.

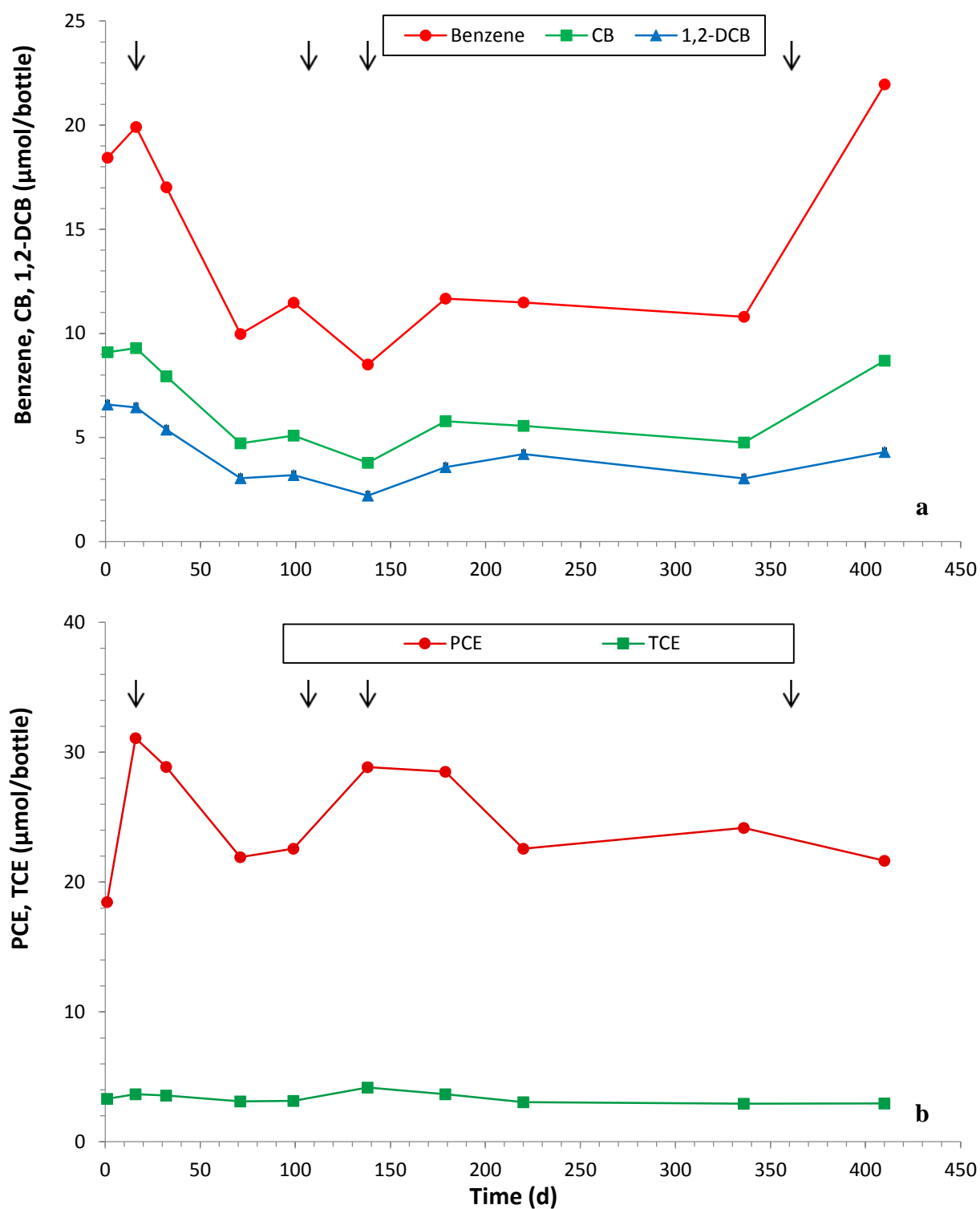


Figure 3.24. Results for the triplicate tank farm anaerobic lactate-amended microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE. Arrows indicate addition of lactate.

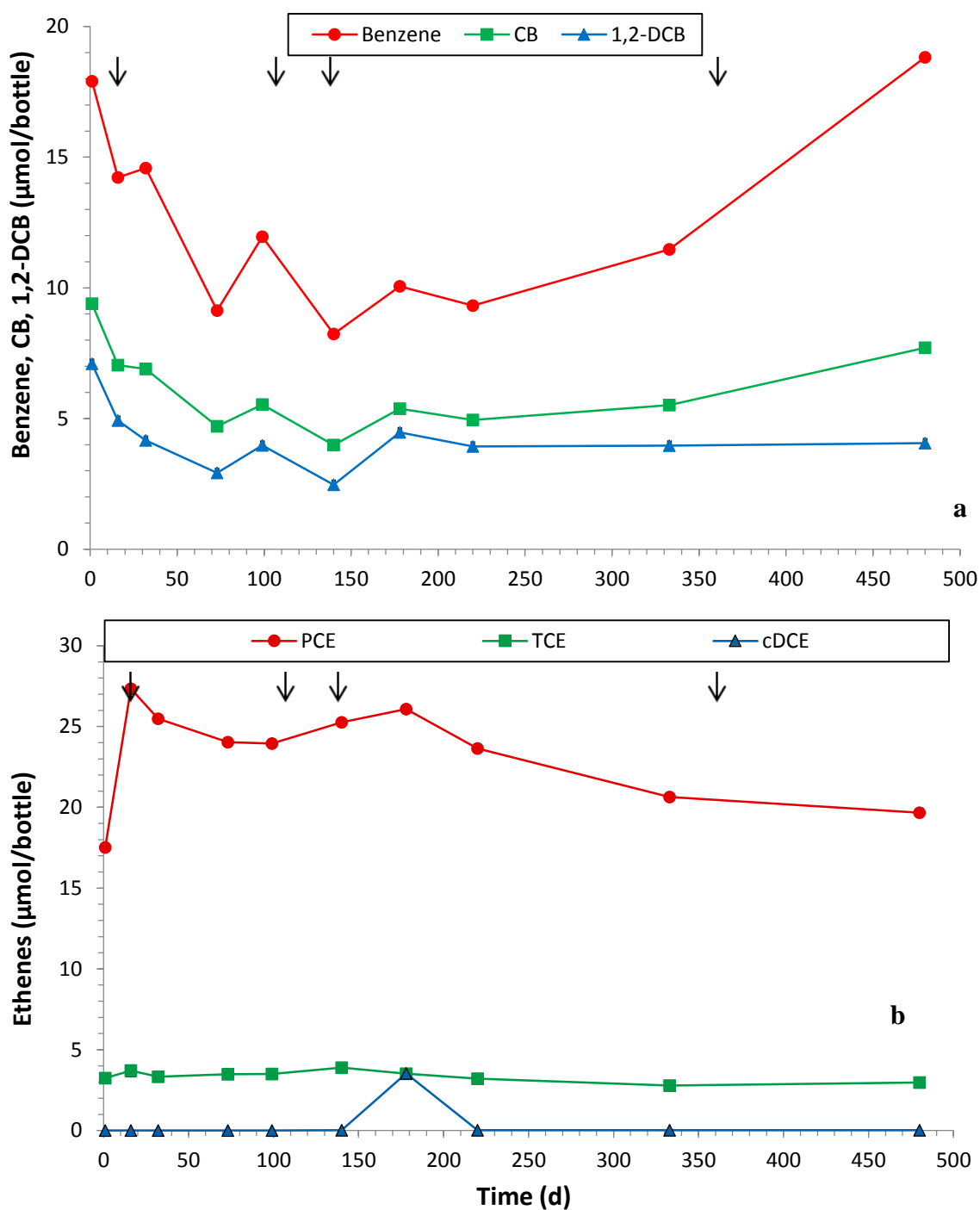


Figure 3.25. Results for the triplicate tank farm EVO-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Arrows indicate addition of EVO.

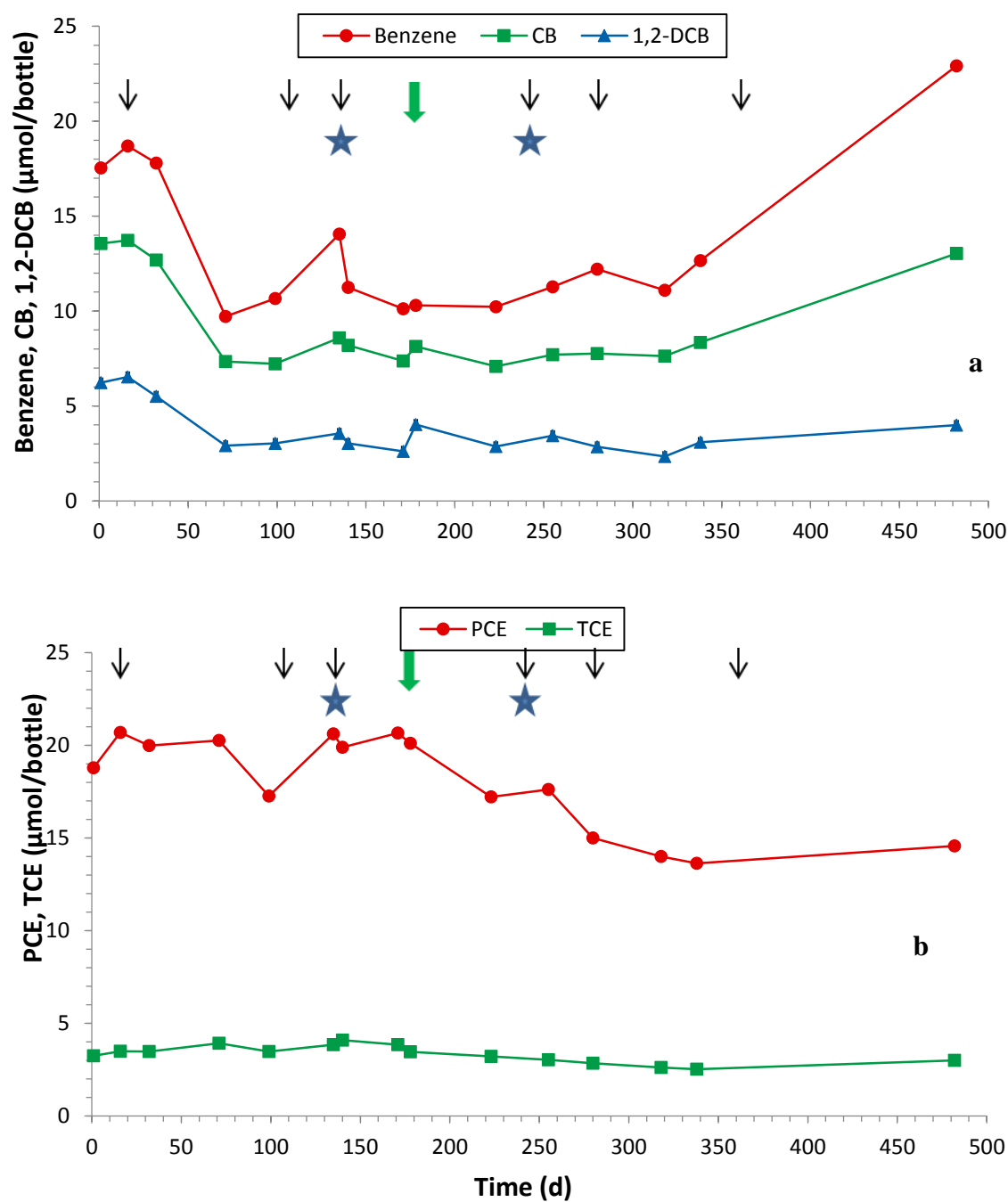


Figure 3.26. Results for the triplicate tank farm bioaugmented anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Black arrows indicate addition of lactate, green arrows indicate addition of corn syrup, and stars indicate addition of the KB-1 bioaugmentation culture.

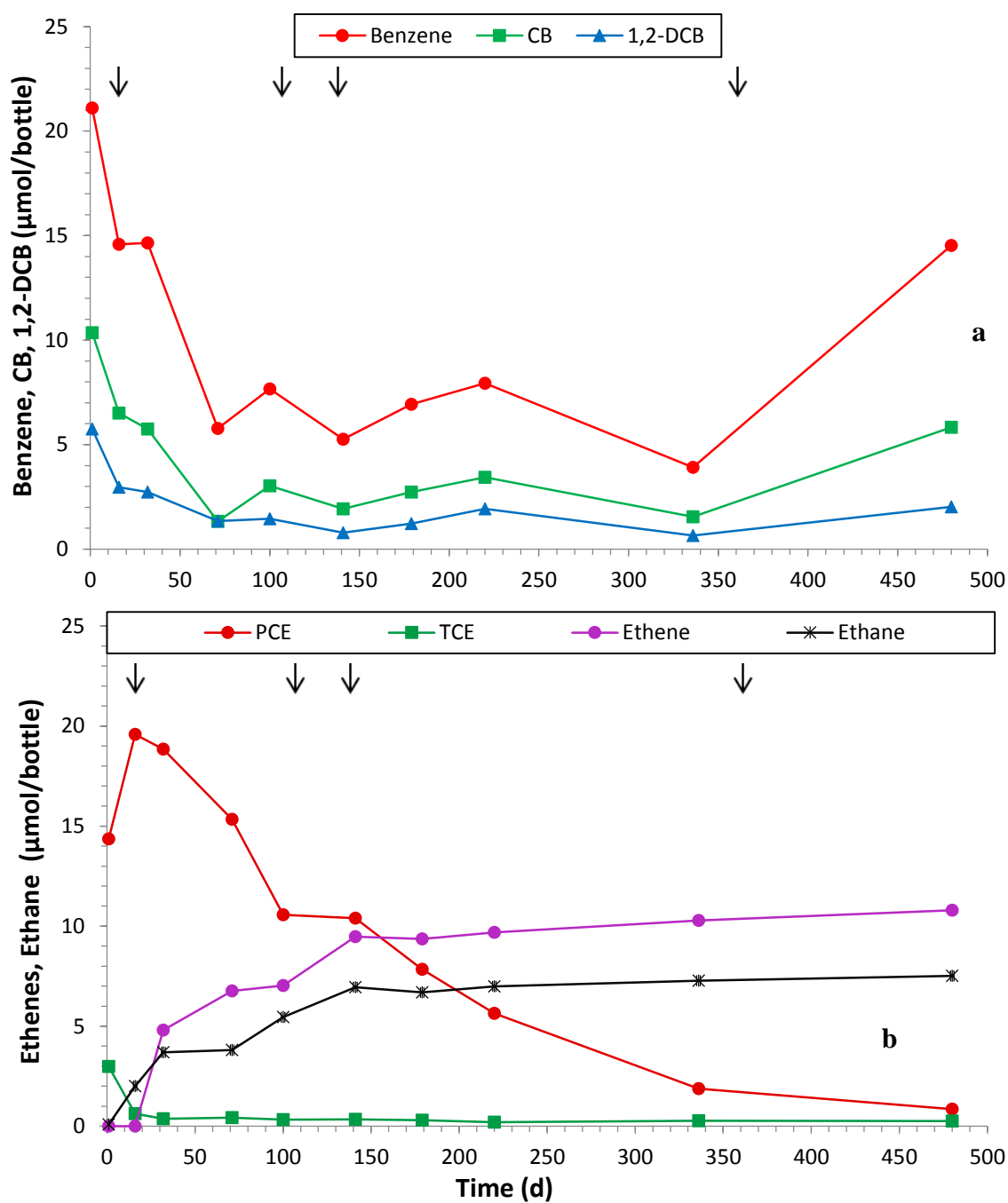


Figure 3.27. Results for the triplicate tank farm ZVI and lactate-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and ethane. Arrows indicate addition of lactate.

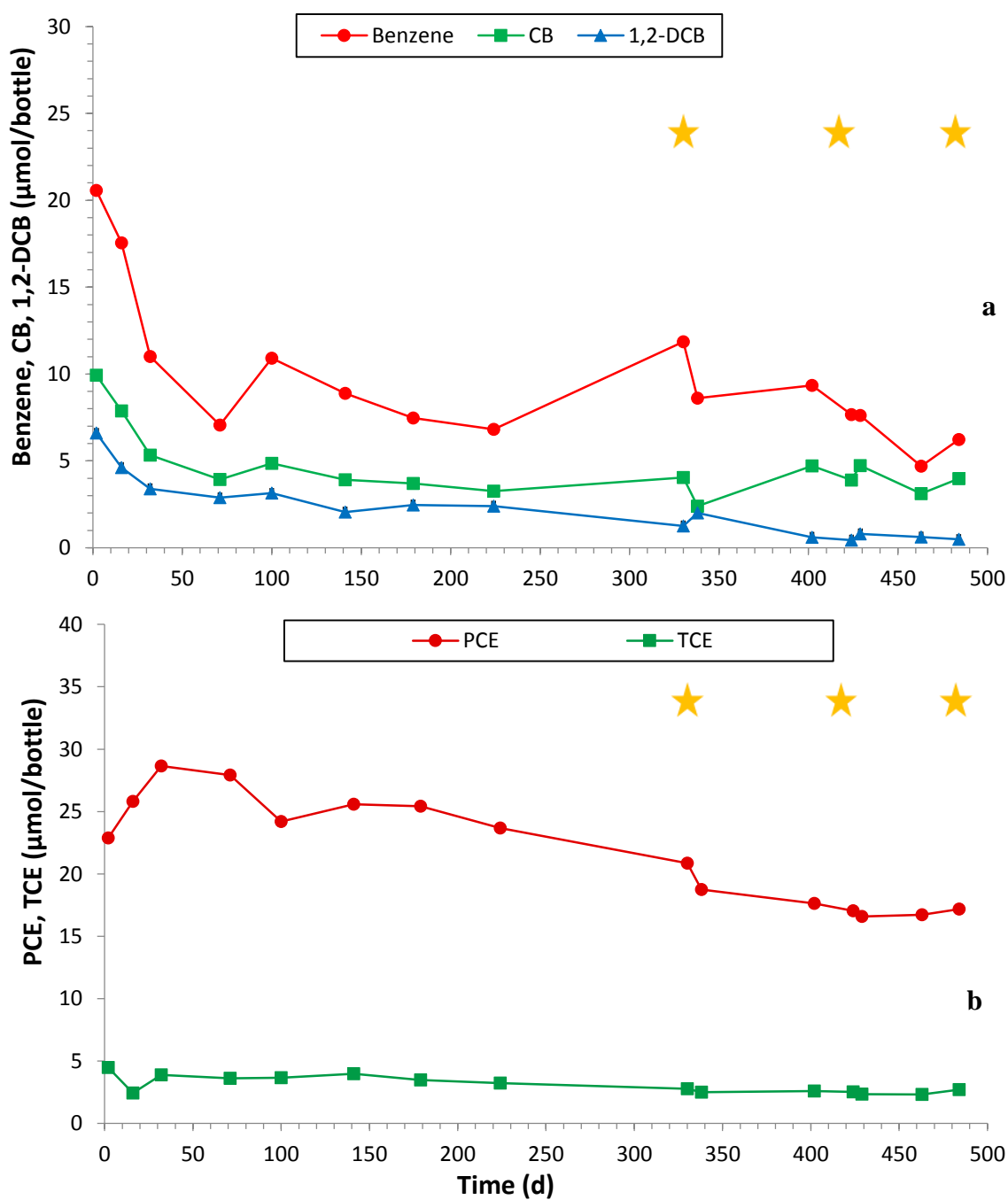


Figure 3.28. Results for the triplicate tank farm unamended aerobic microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE. Stars indicate addition of 2.0 mL from the WWTP AER microcosm #2.

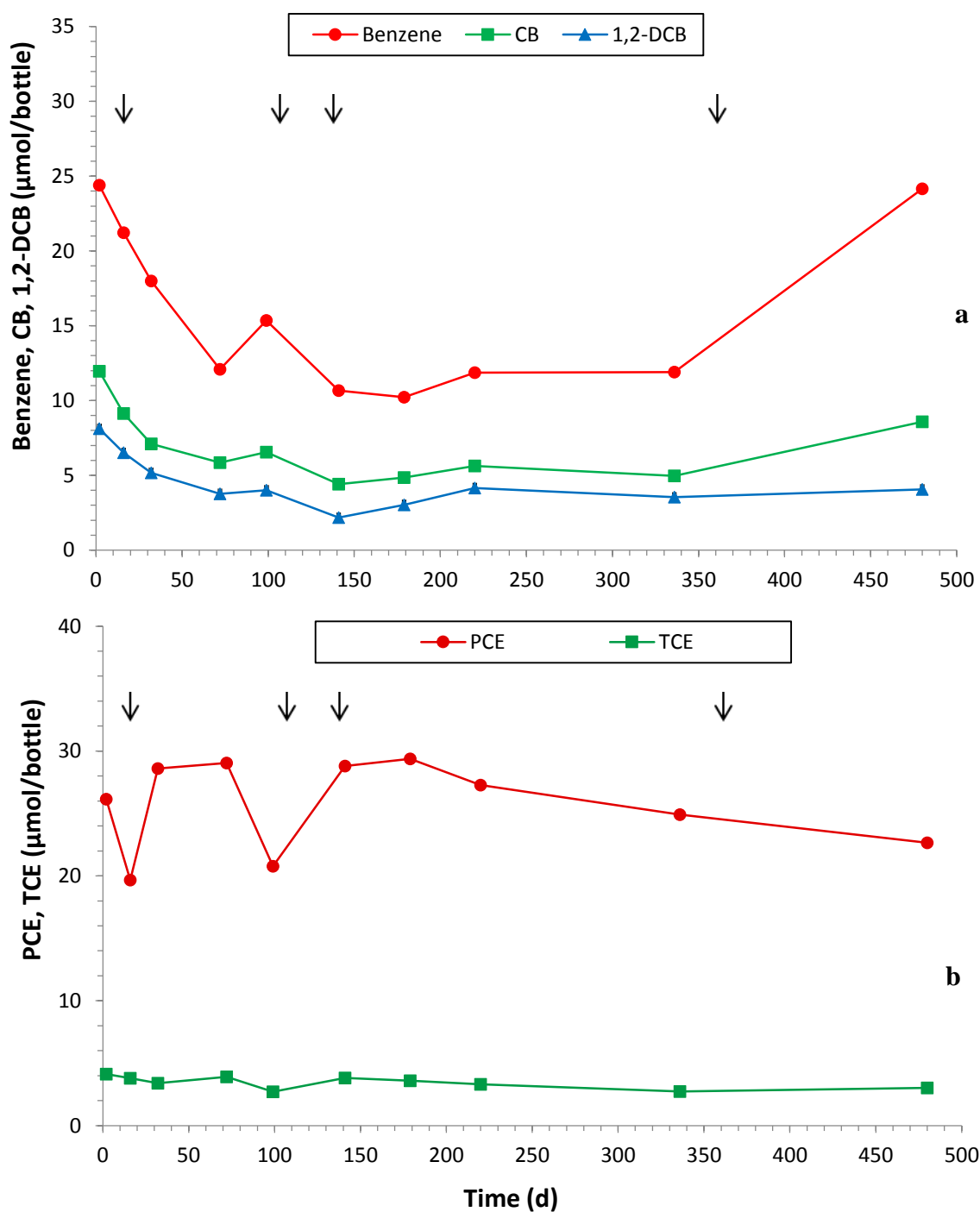


Figure 3.29. Results for the triplicate tank farm lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.

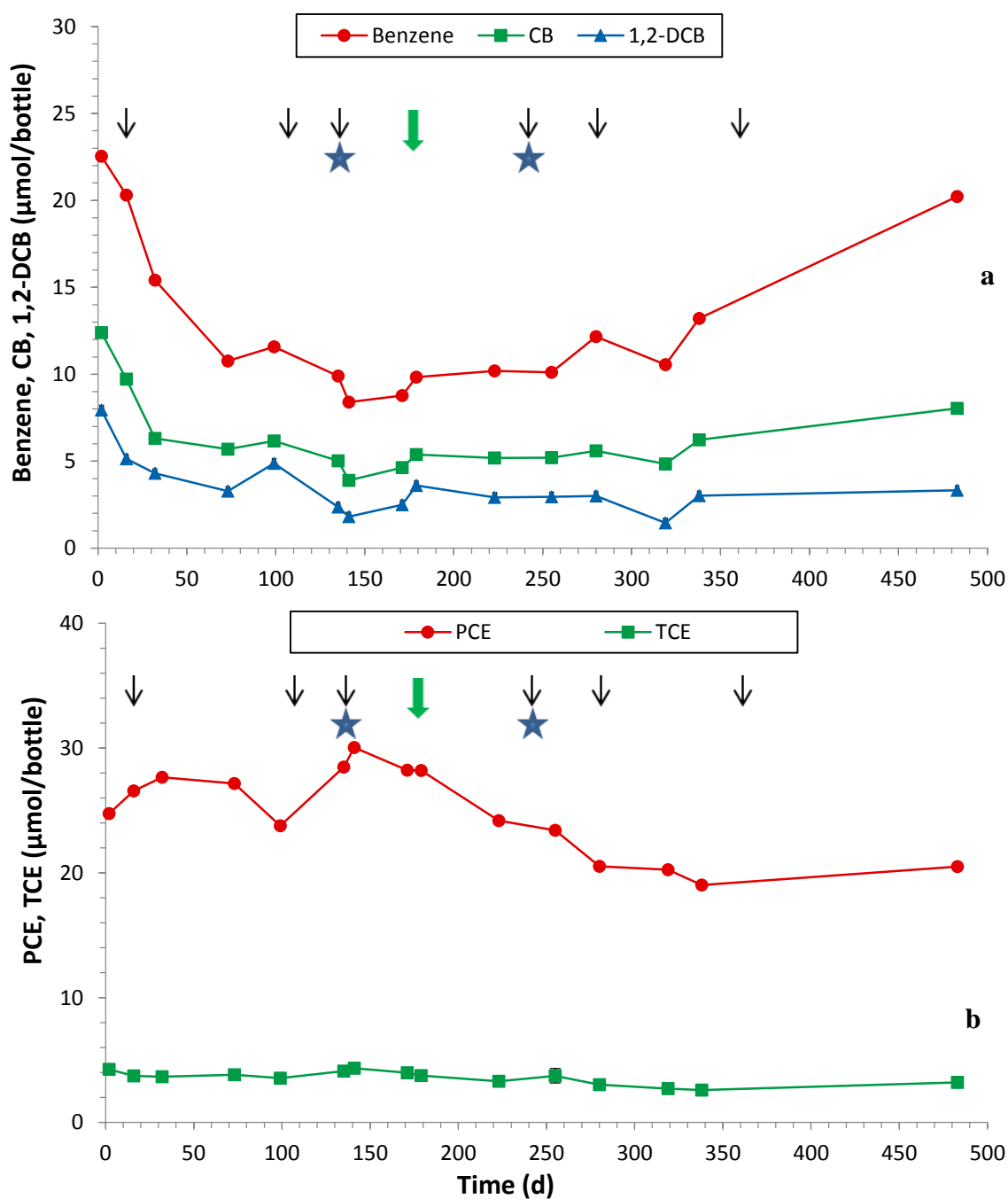


Figure 3.30. Results for the triplicate tank farm bioaugmented anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) PCE and TCE. Black arrows indicate addition of lactate, green arrows indicate addition of corn syrup and stars indicate addition of KB-1 culture. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.

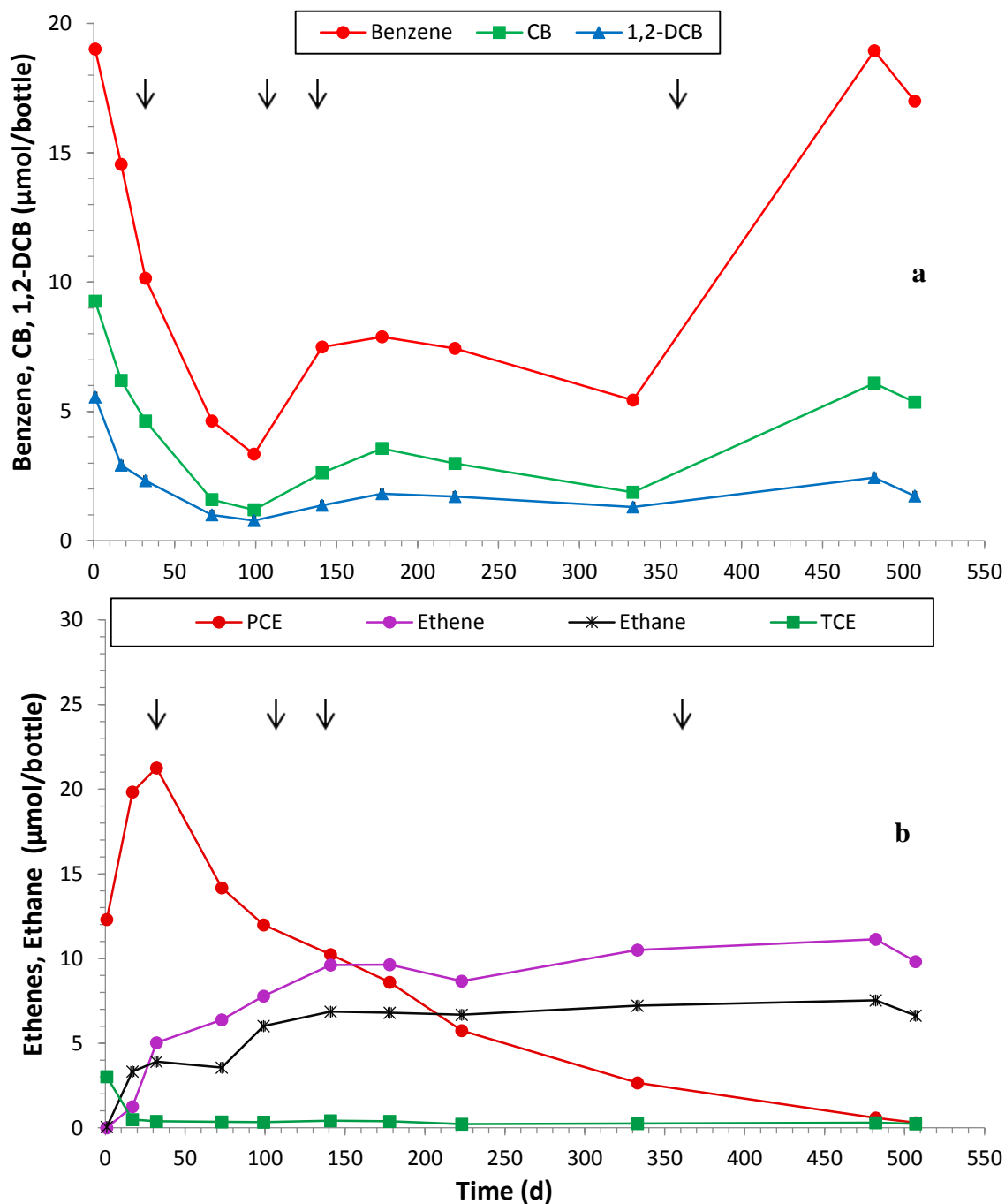


Figure 3.31. Results for the tank farm ZVI and lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and ethane. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.

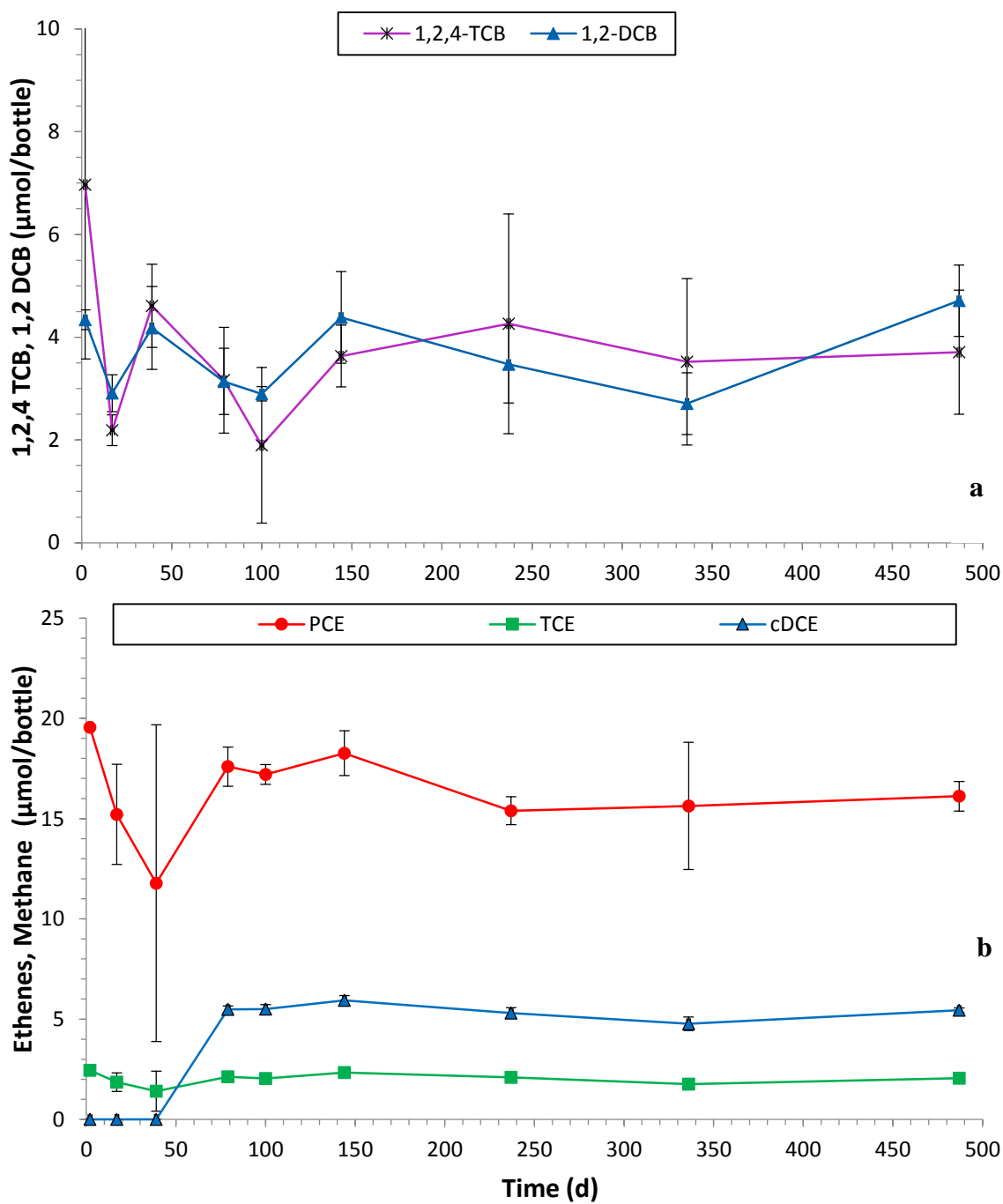


Figure 3.32. Results for the triplicate grease trap autoclaved control microcosms; error bars represent standard deviations; a) aromatic compounds at high concentrations; and b) PCE and TCE.

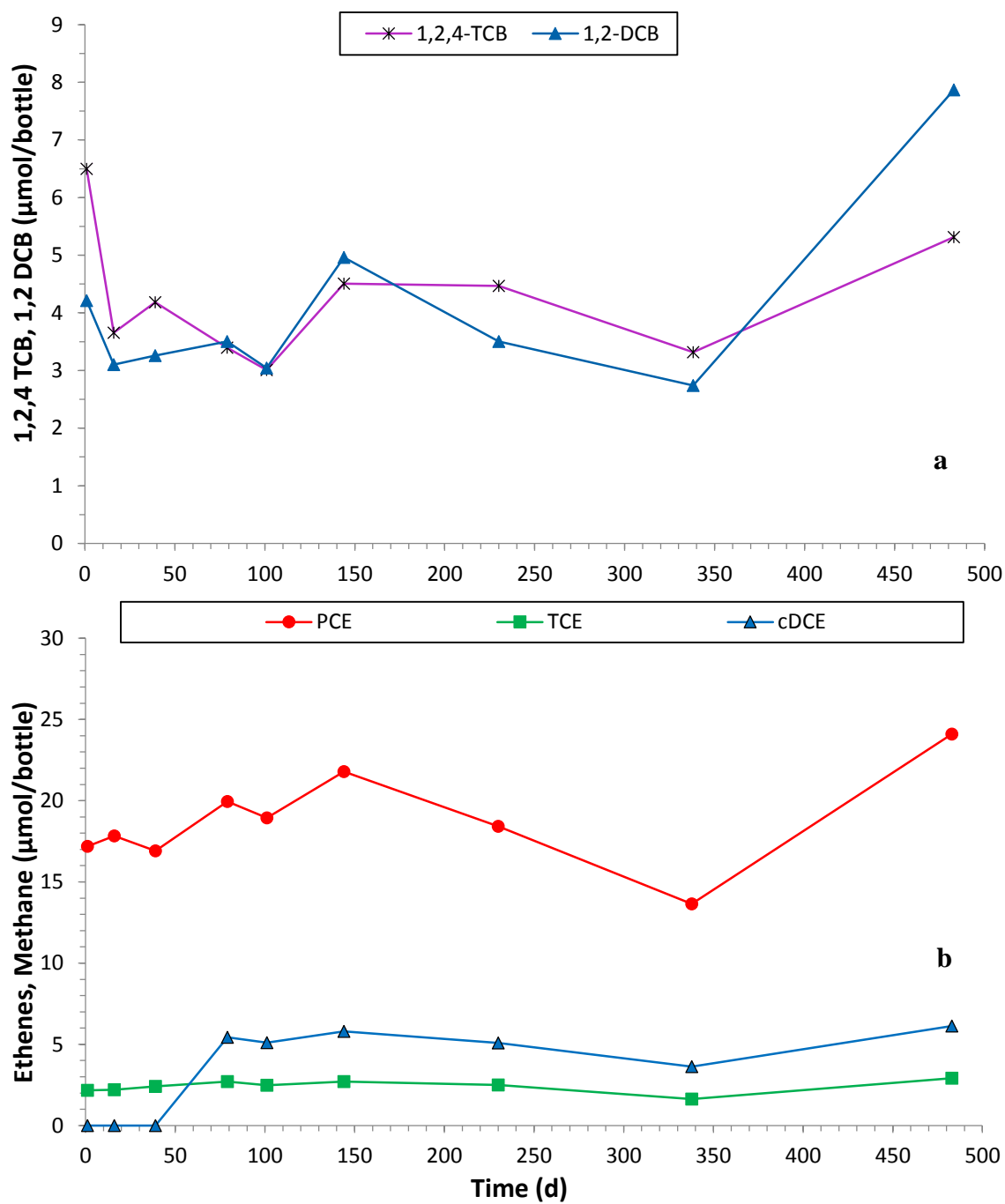
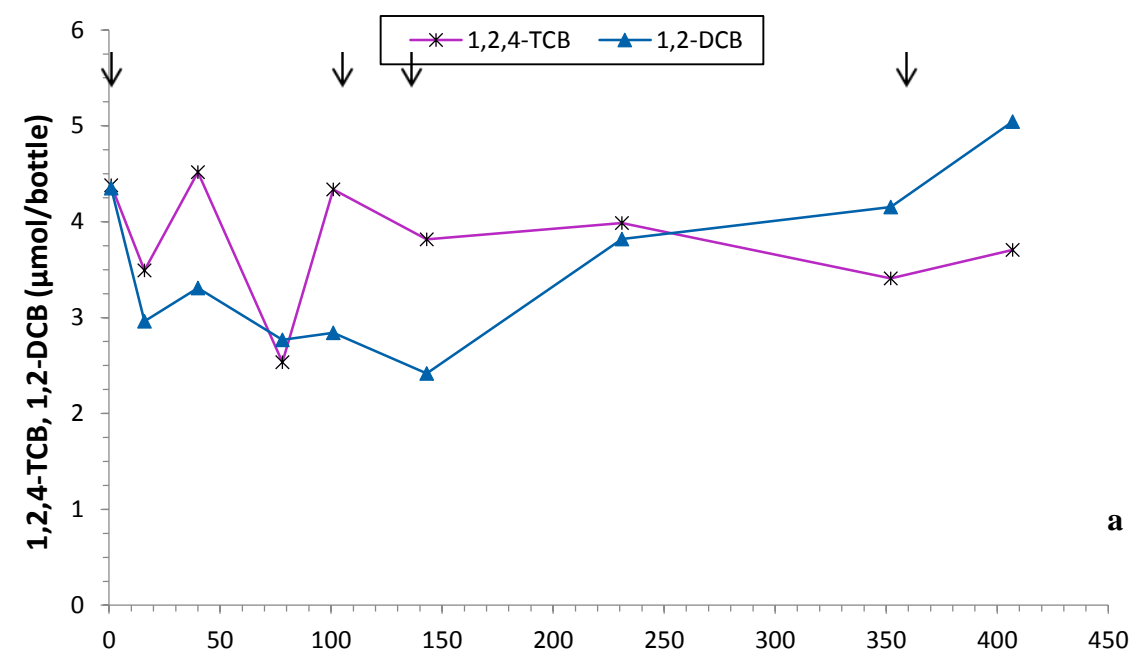
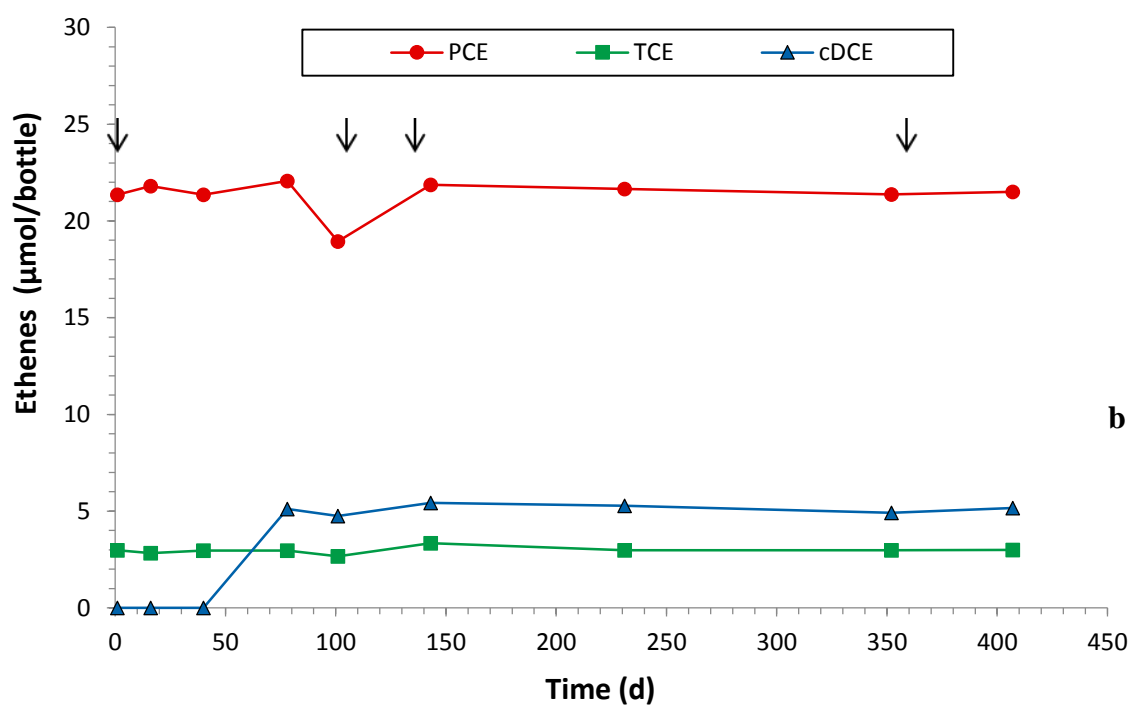


Figure 3.33. Results for the triplicate grease trap unamended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes.



a



b

Figure 3.34. Results for the triplicate grease trap anaerobic lactate-amended microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Arrows indicate addition of lactate.

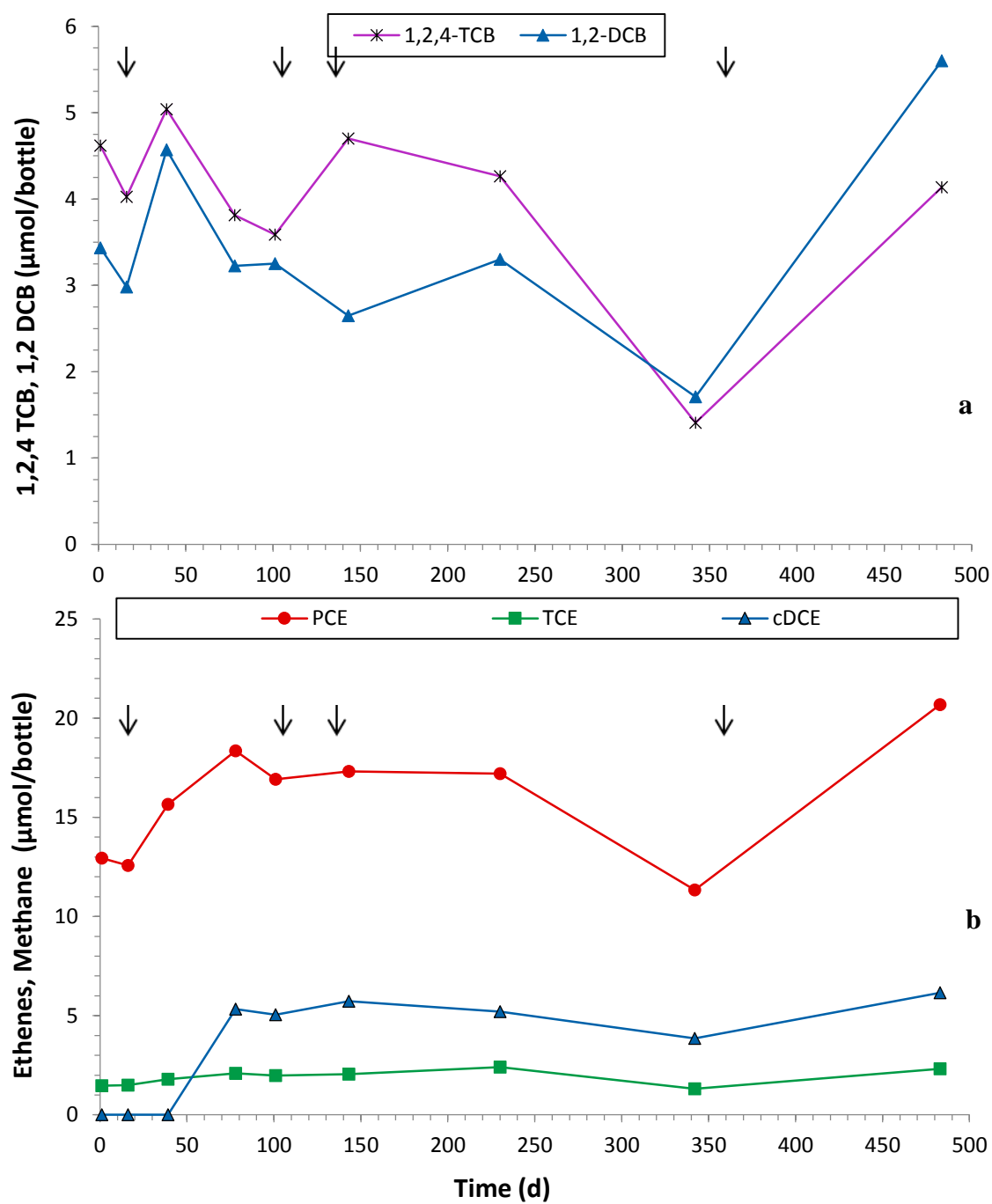


Figure 3.35. Results for the triplicate grease trap EVO-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Arrows indicate addition of EVO.

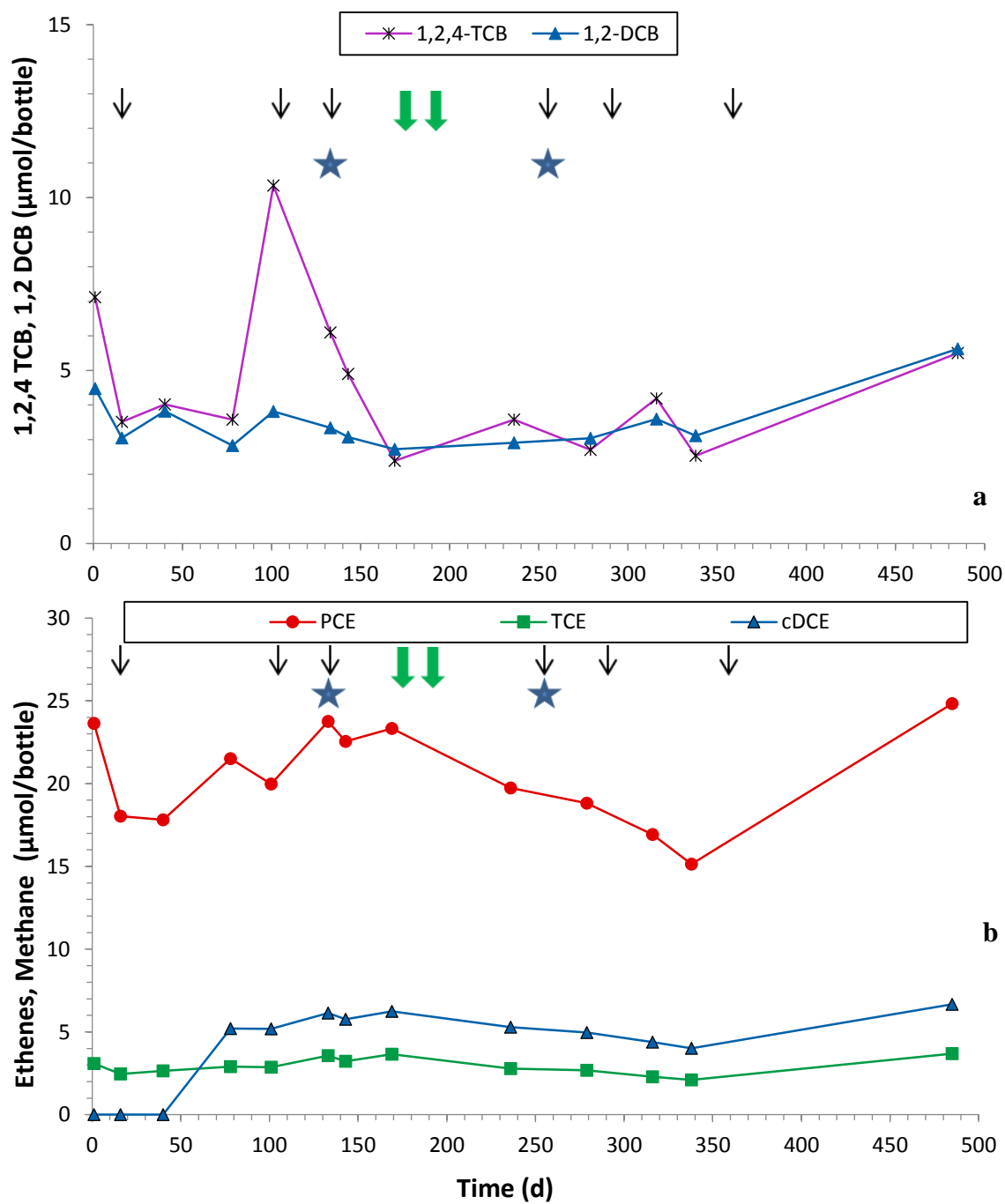


Figure 3.36. Results for the triplicate grease trap bioaugmented anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Black arrows indicate addition of lactate, green arrows indicate addition of corn syrup and stars indicate addition of KB-1 culture.

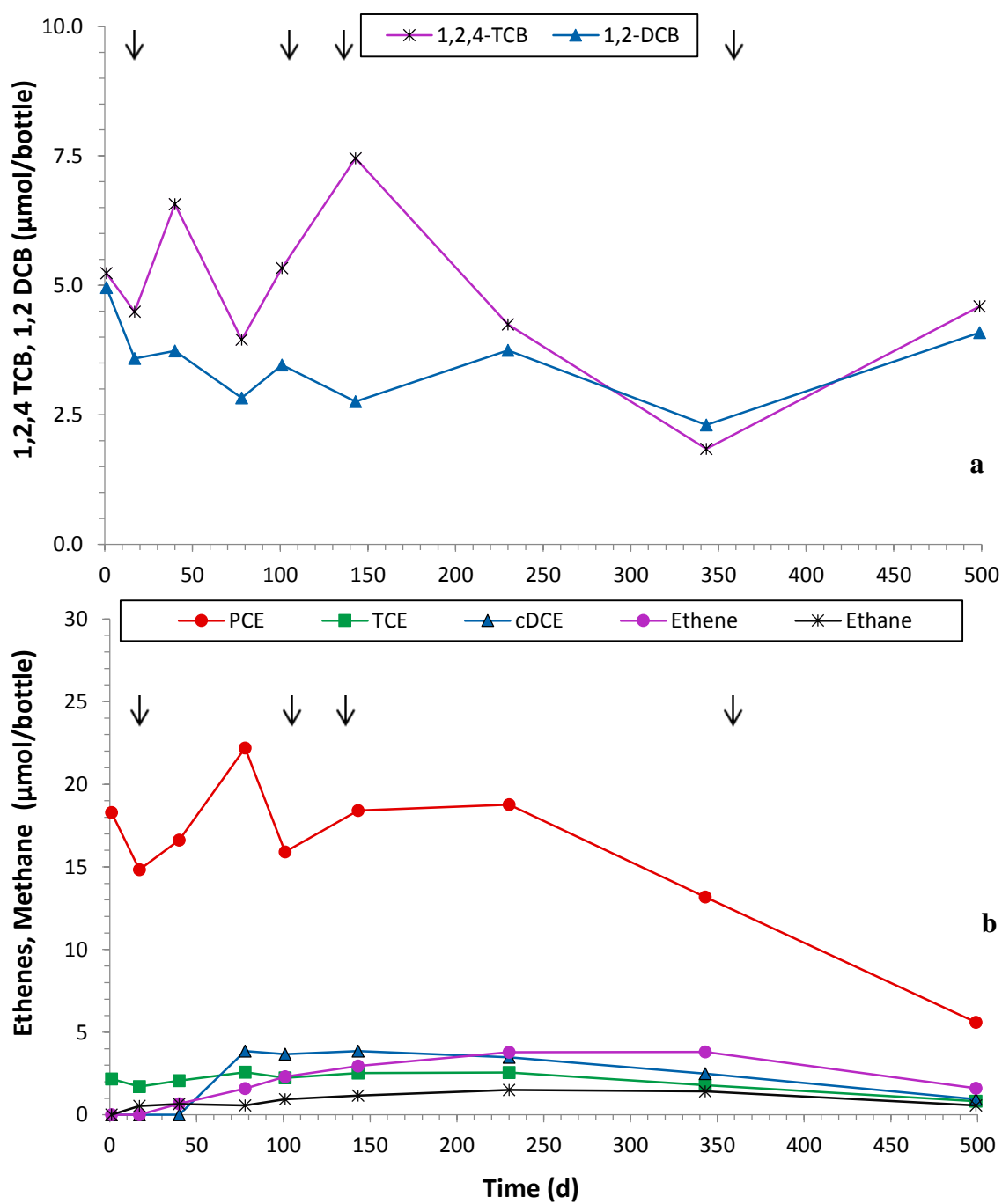


Figure 3.37. Results for the triplicate grease trap ZVI and lactate-amended anaerobic microcosms; a) chlorinated benzenes at high concentrations; and b) chlorinated ethenes, ethene, and ethane. Arrows indicate addition of lactate.

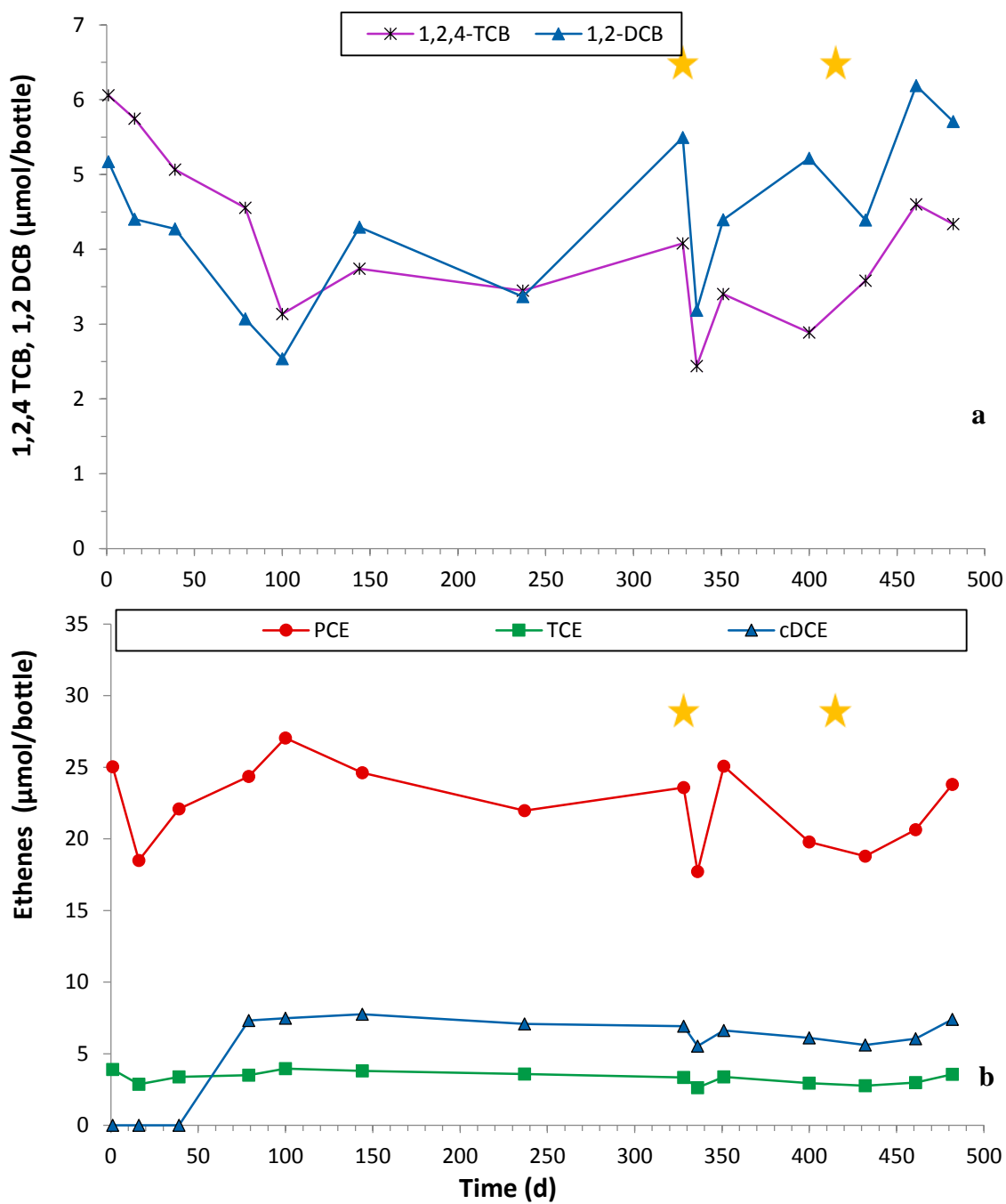


Figure 3.38. Results for the triplicate grease trap unamended aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Stars indicate addition of 2.0 mL from the WWTP AER microcosm #2.

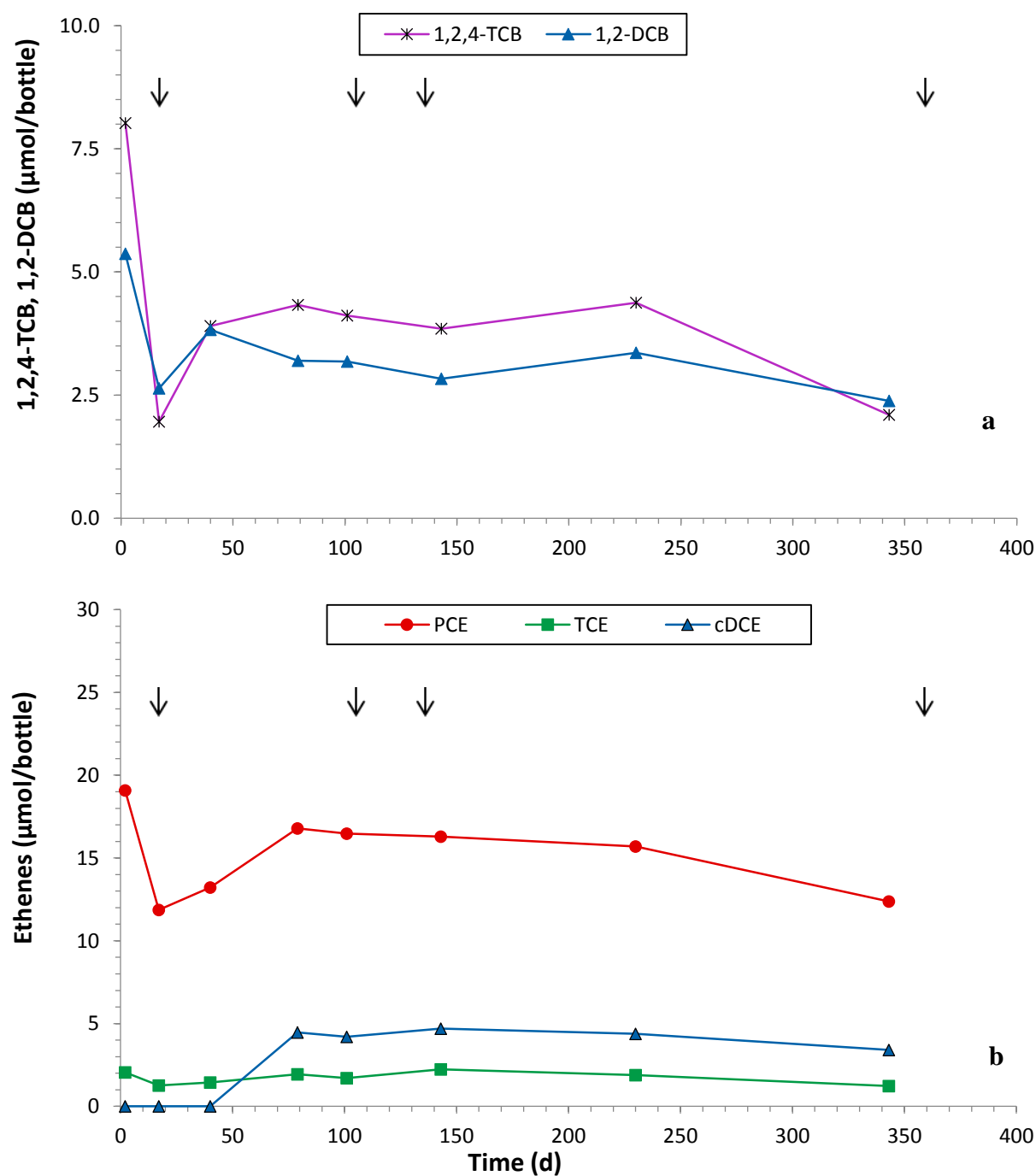


Figure 3.39. Results for the triplicate grease trap lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.

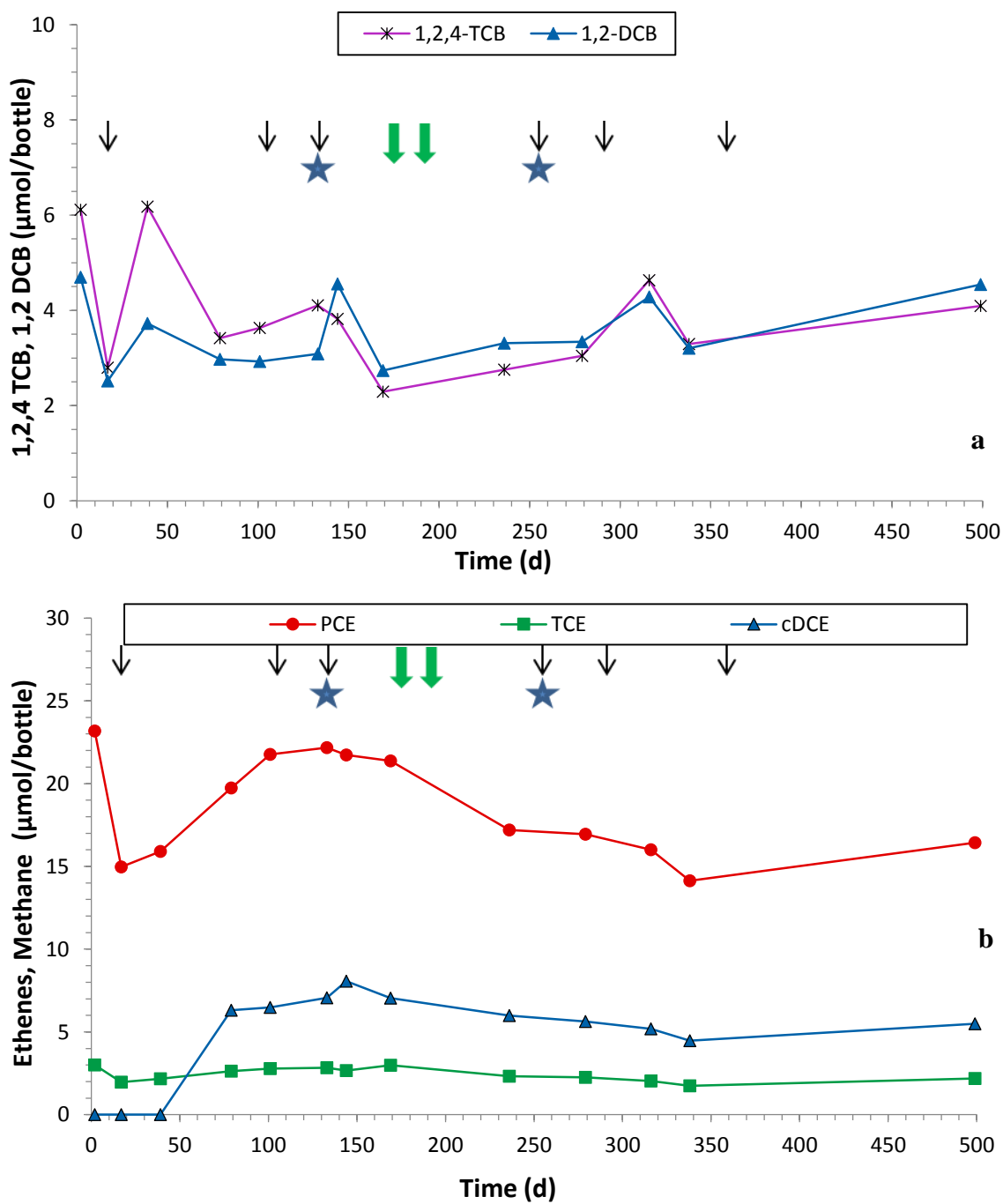


Figure 3.40. Results for the triplicate grease trap bioaugmented anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes. Black arrows indicate addition of lactate, green arrows indicate addition of corn syrup and stars indicate addition of KB-1 culture. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.

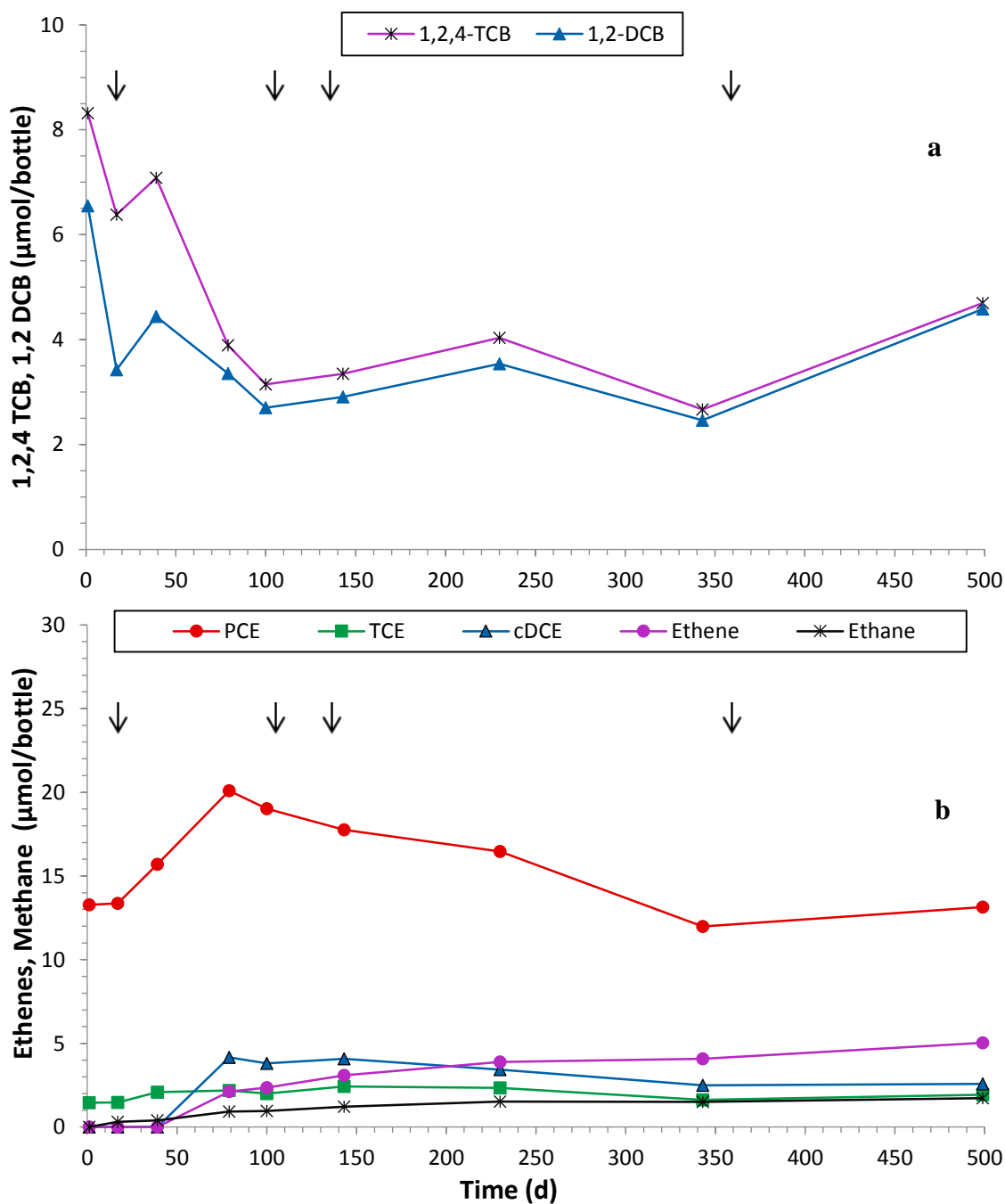


Figure 3.41. Results for the grease trap ZVI and lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and ethane. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.

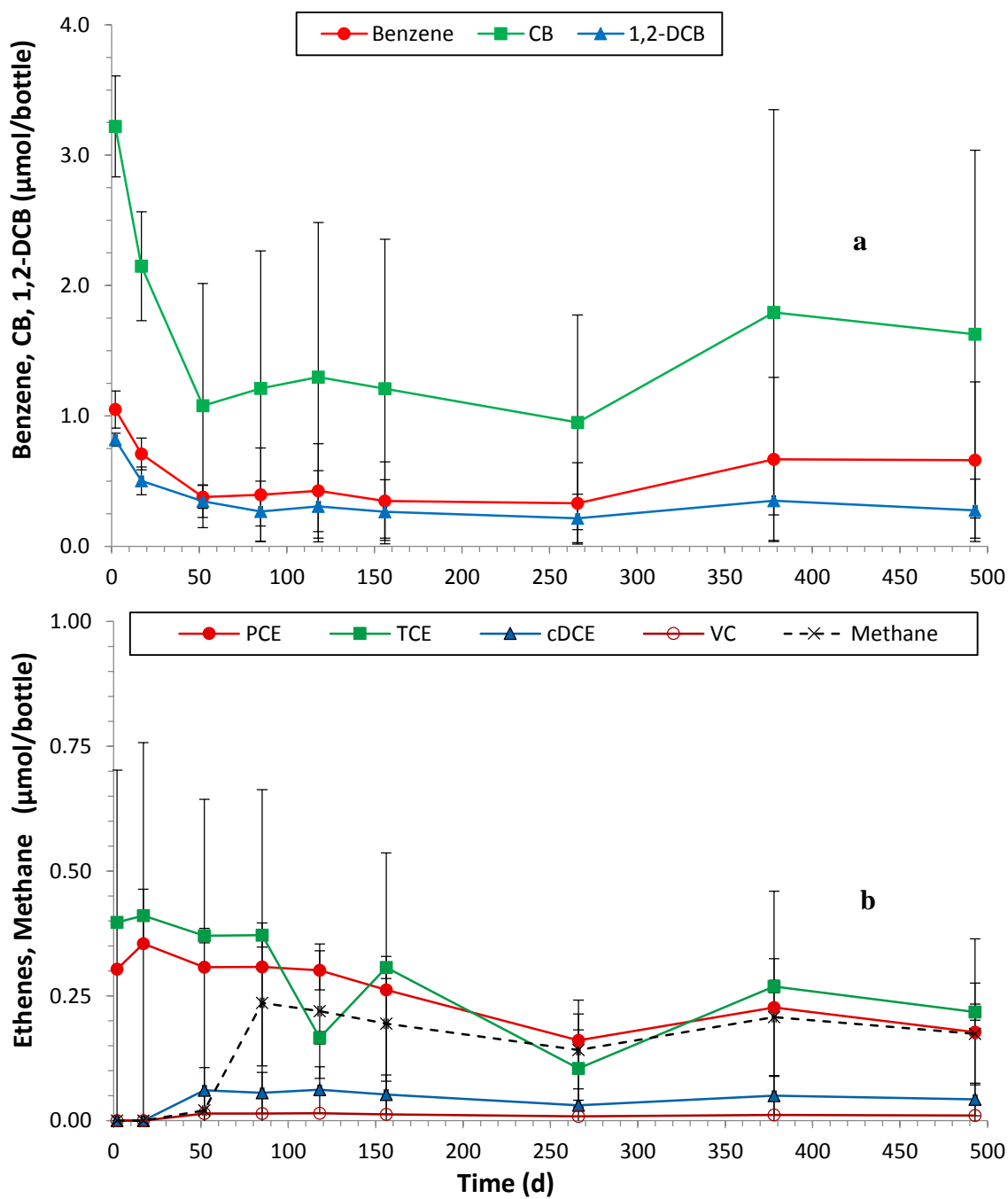


Figure 3.42. Results for the triplicate downgradient plume autoclaved control microcosms; error bars represent standard deviations; a) aromatic compounds; and b) chlorinated ethenes.

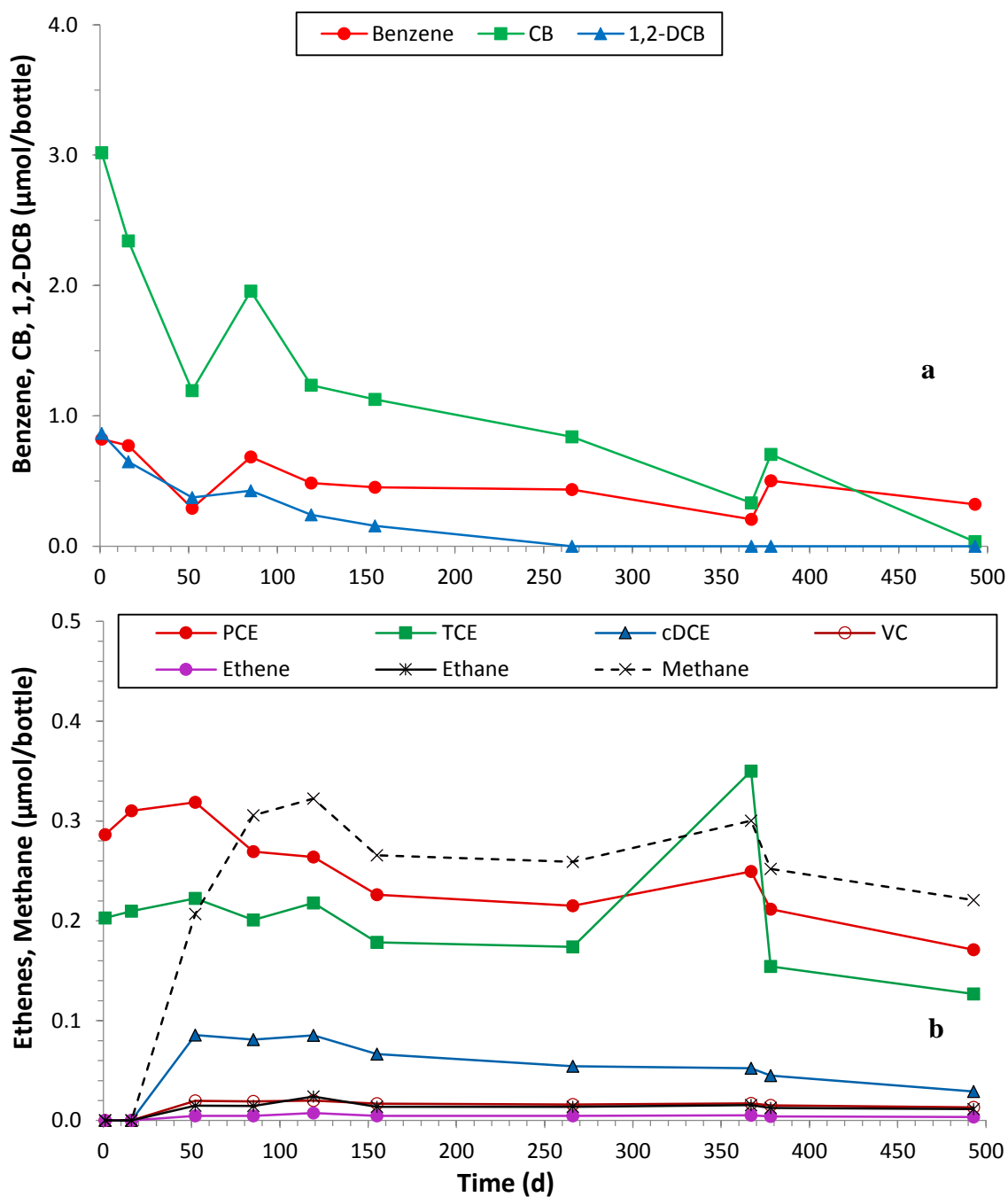


Figure 3.43. Results for the triplicate downgradient plume unamended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene and methane.

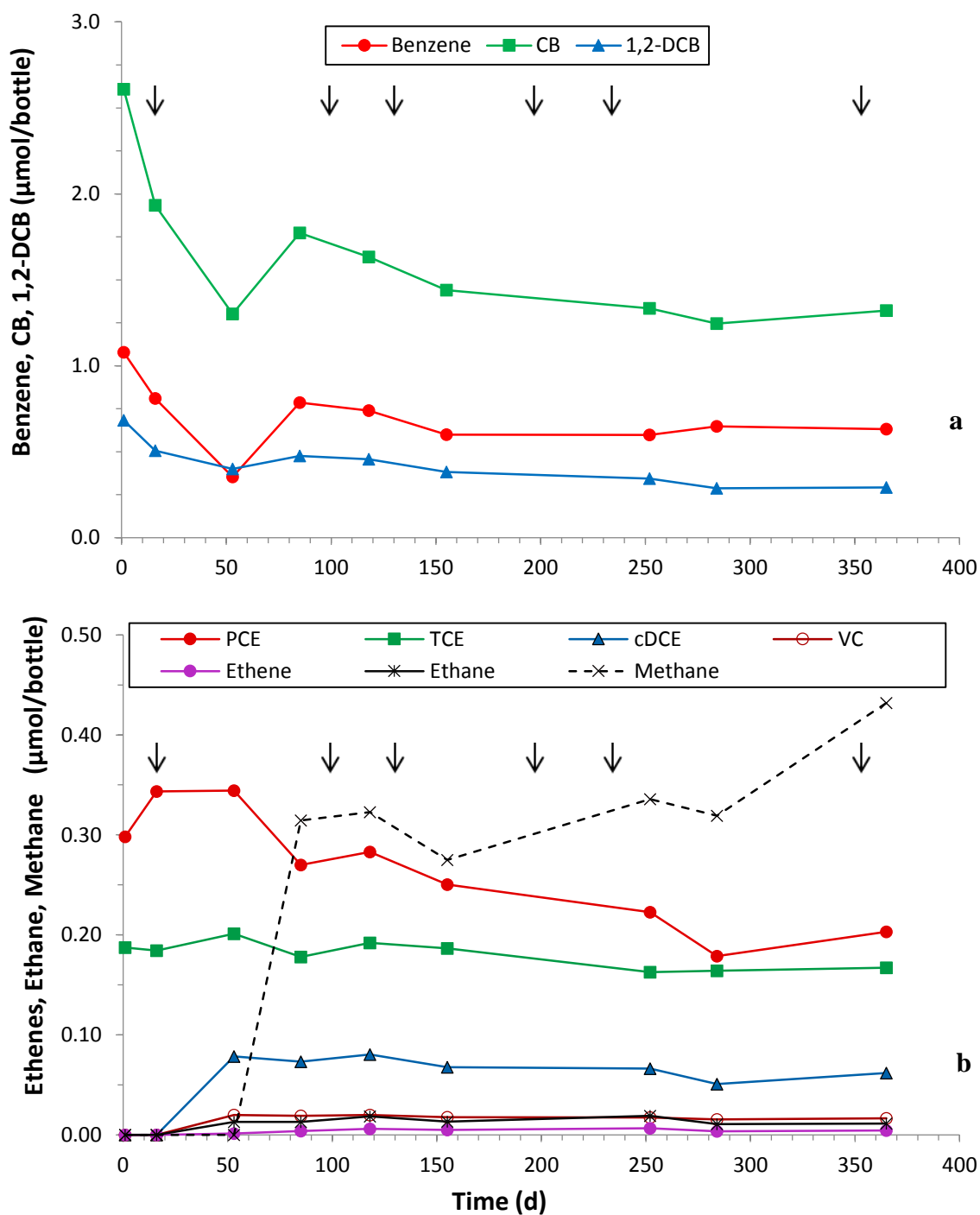


Figure 3.44. Results for the triplicate downgradient plume anaerobic lactate-amended microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate.

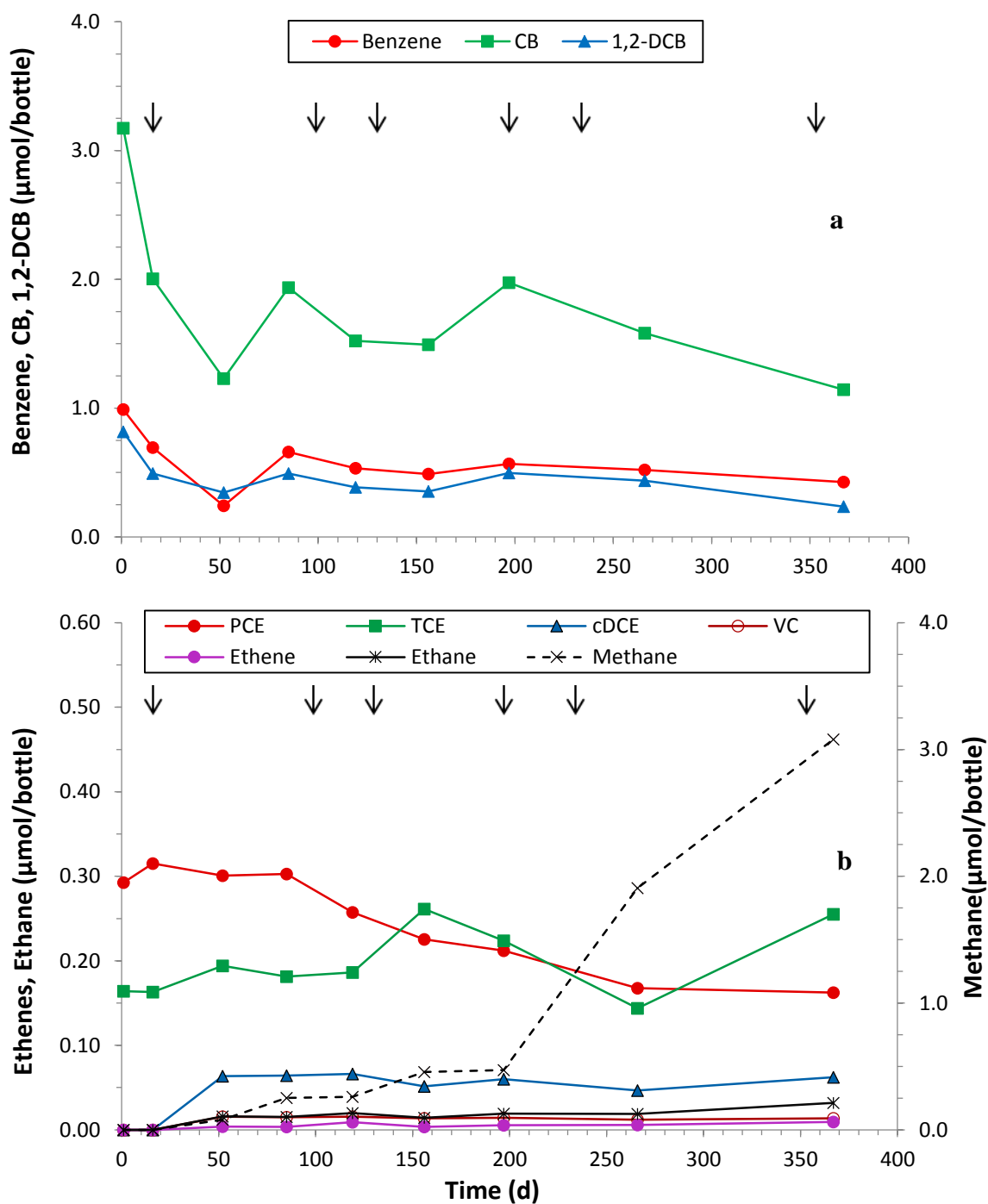


Figure 3.45. Results for the triplicate downgradient plume EVO-amended anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of EVO.

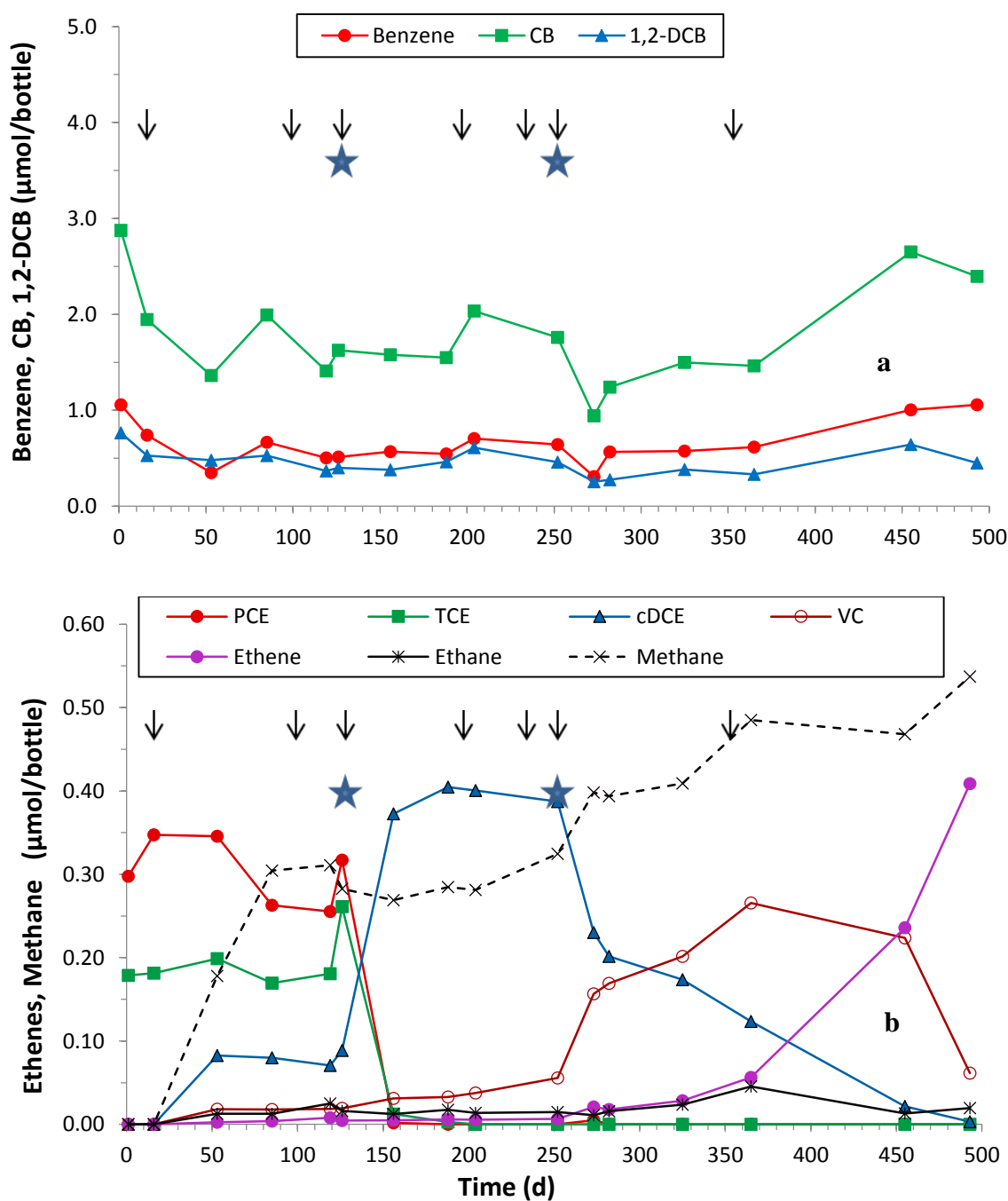


Figure 3.46. Results for the triplicate downgradient plume bioaugmented anaerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene, and methane. Arrows indicate addition of lactate and stars indicate addition of the KB-1 bioaugmentation culture.

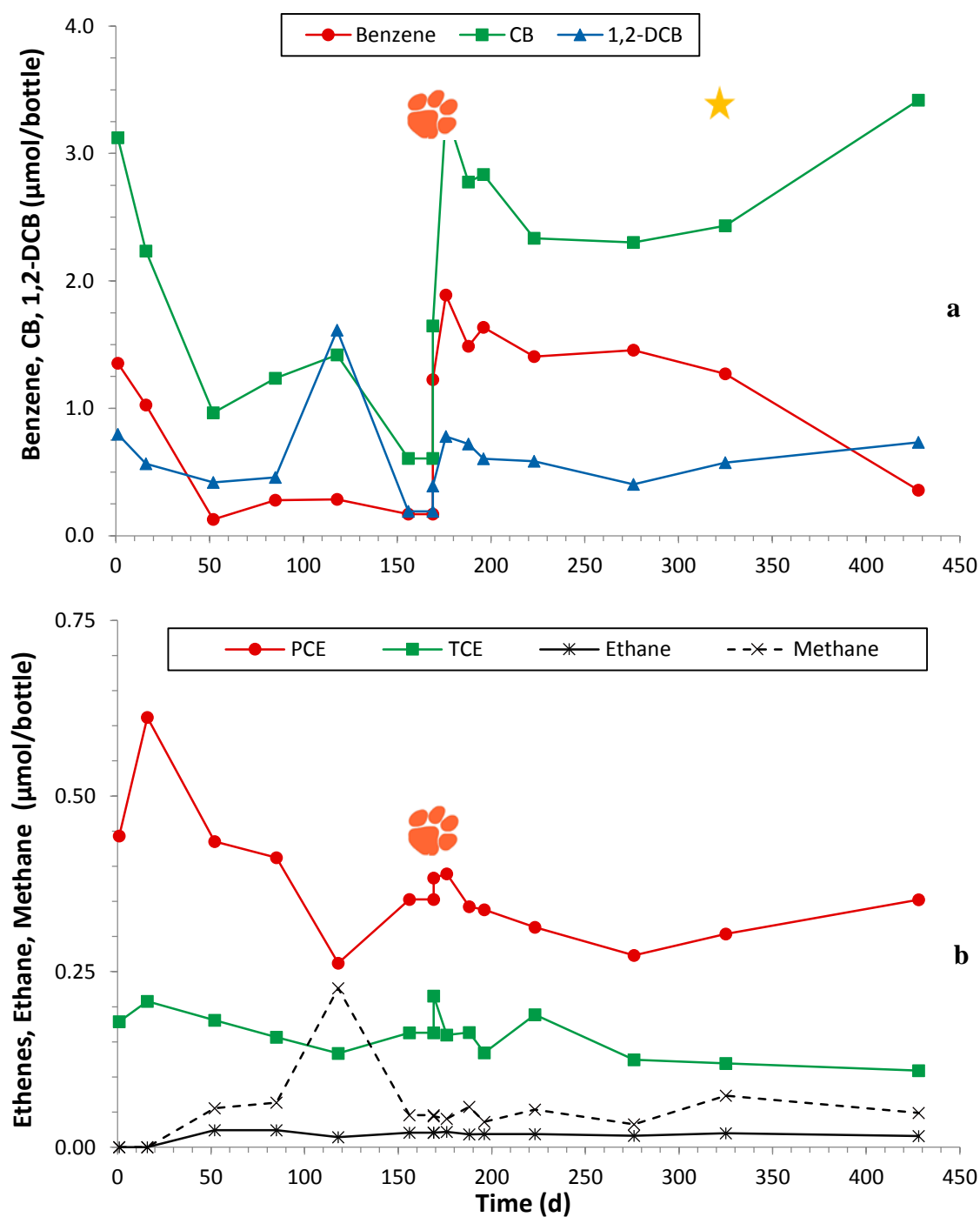


Figure 3.47. Results for downgradient plume unamended aerobic microcosm #1; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethane, and methane. Tiger paws indicate addition of aromatic compounds.

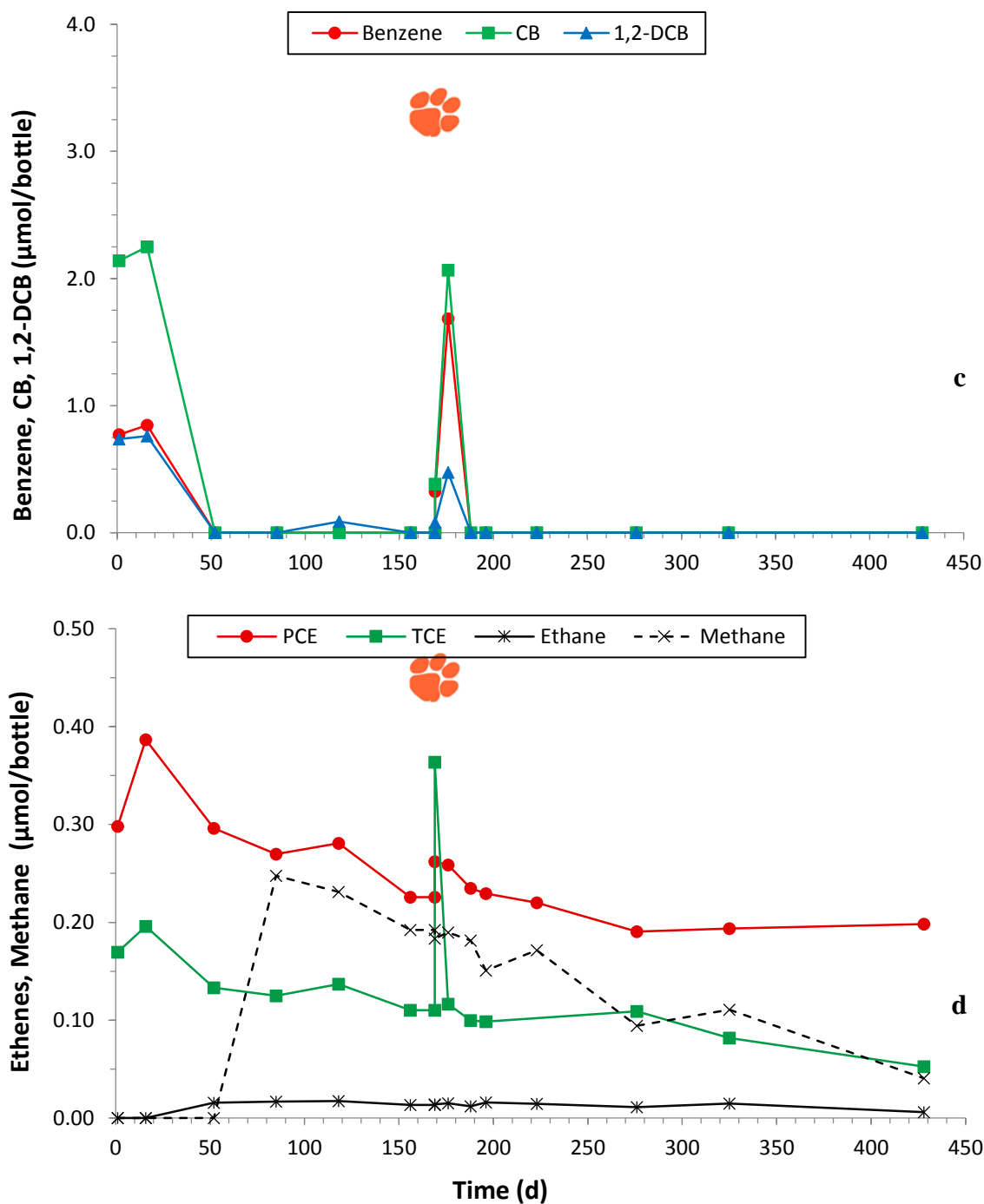


Figure 3.47 (continued). Results for downgradient plume unamended aerobic microcosm #2; c) aromatic compounds at high concentrations; and d) chlorinated ethenes, ethane, and methane. Tiger paws indicate addition of aromatic compounds.

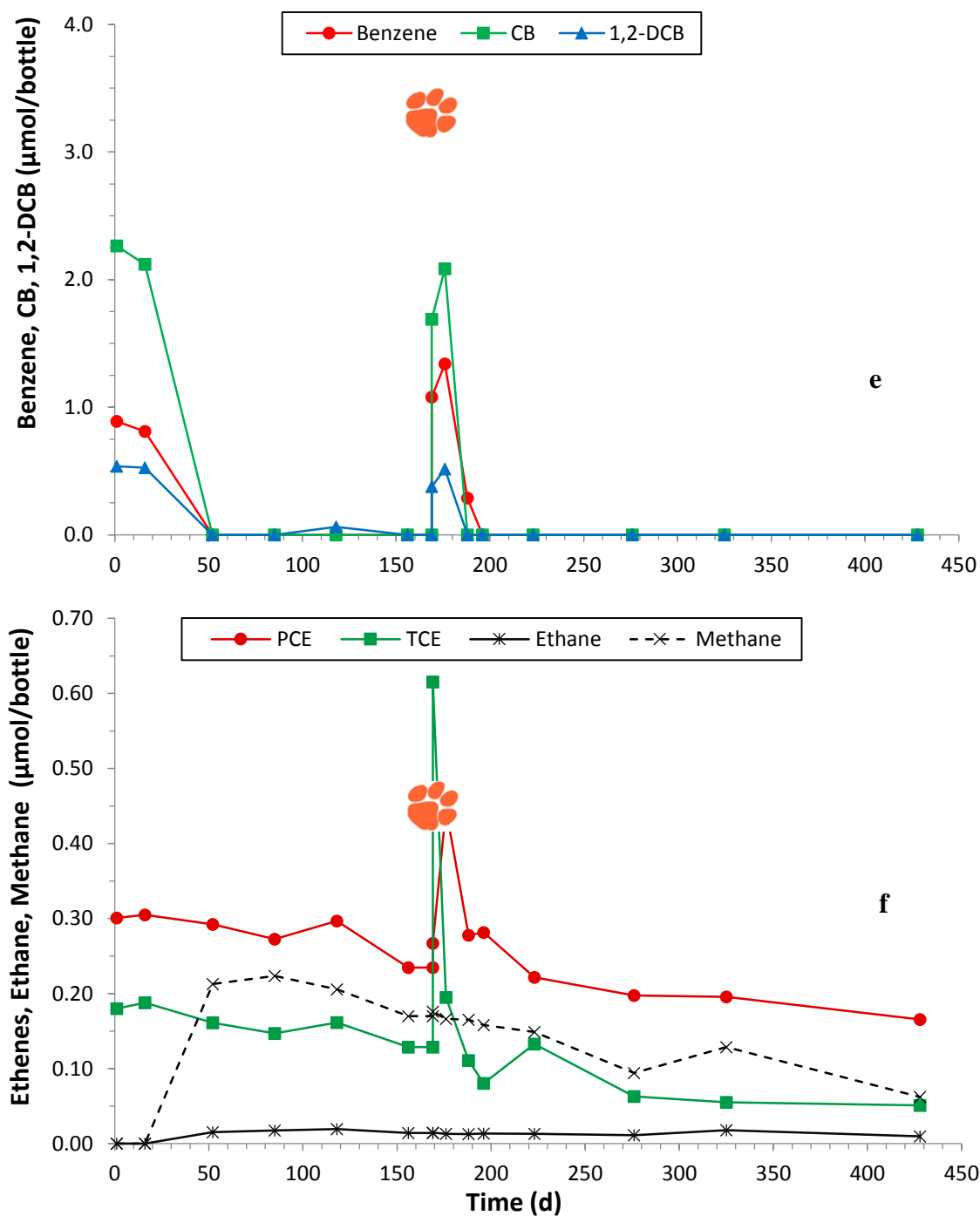


Figure 3.47 (continued). Results for downgradient plume unamended aerobic microcosm #3; e) aromatic compounds at high concentrations; and f) chlorinated ethenes, ethane, and methane. Tiger paws indicate addition of aromatic compounds.

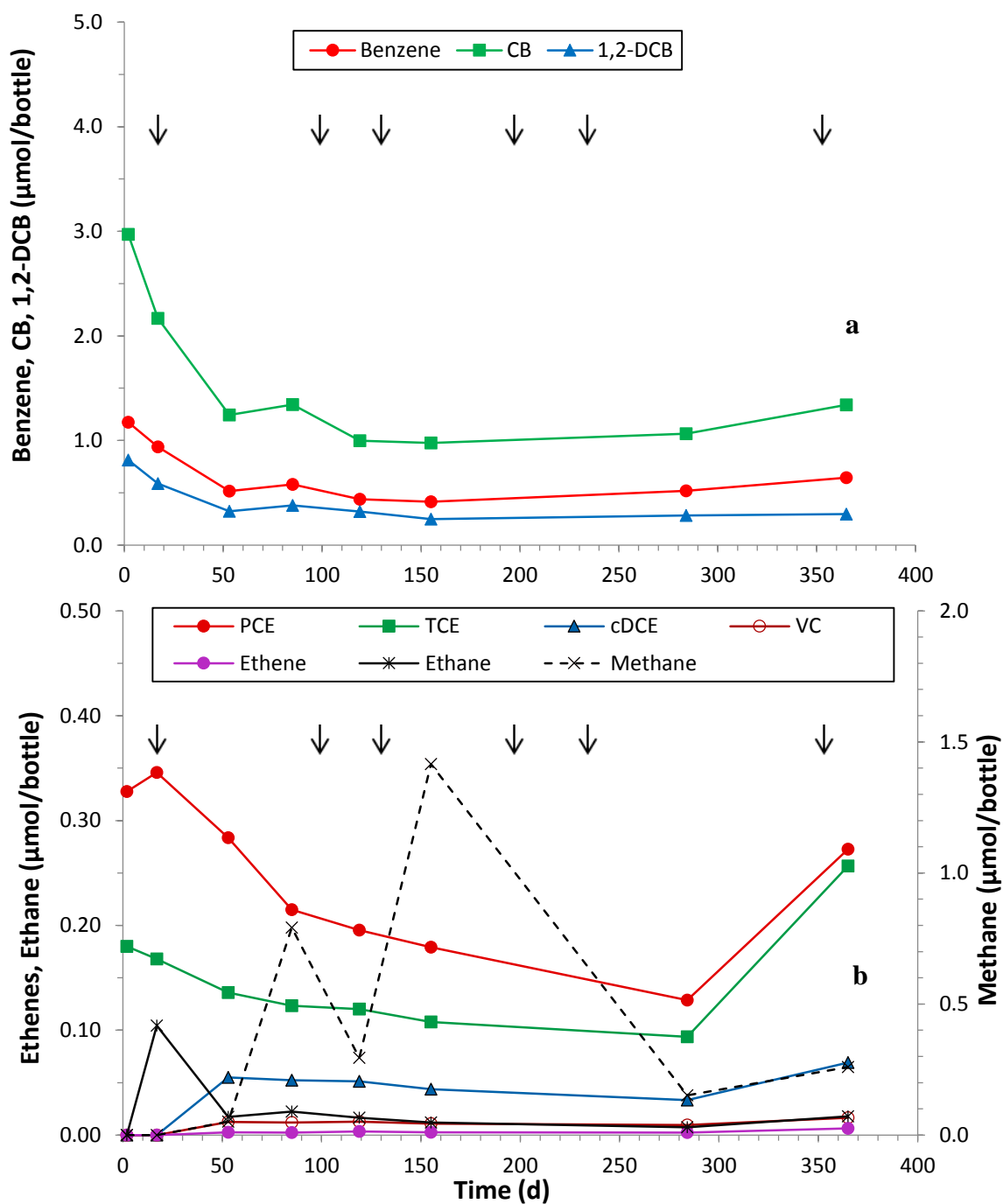


Figure 3.48. Results for the triplicate downgradient plume lactate-amended anaerobic/aerobic microcosms; a) aromatic compounds at high concentrations; and b) chlorinated ethenes, ethene and methane. Arrows indicate addition of lactate. Due to a lack of activity on PCE and TCE, the switch to aerobic conditions was not made.

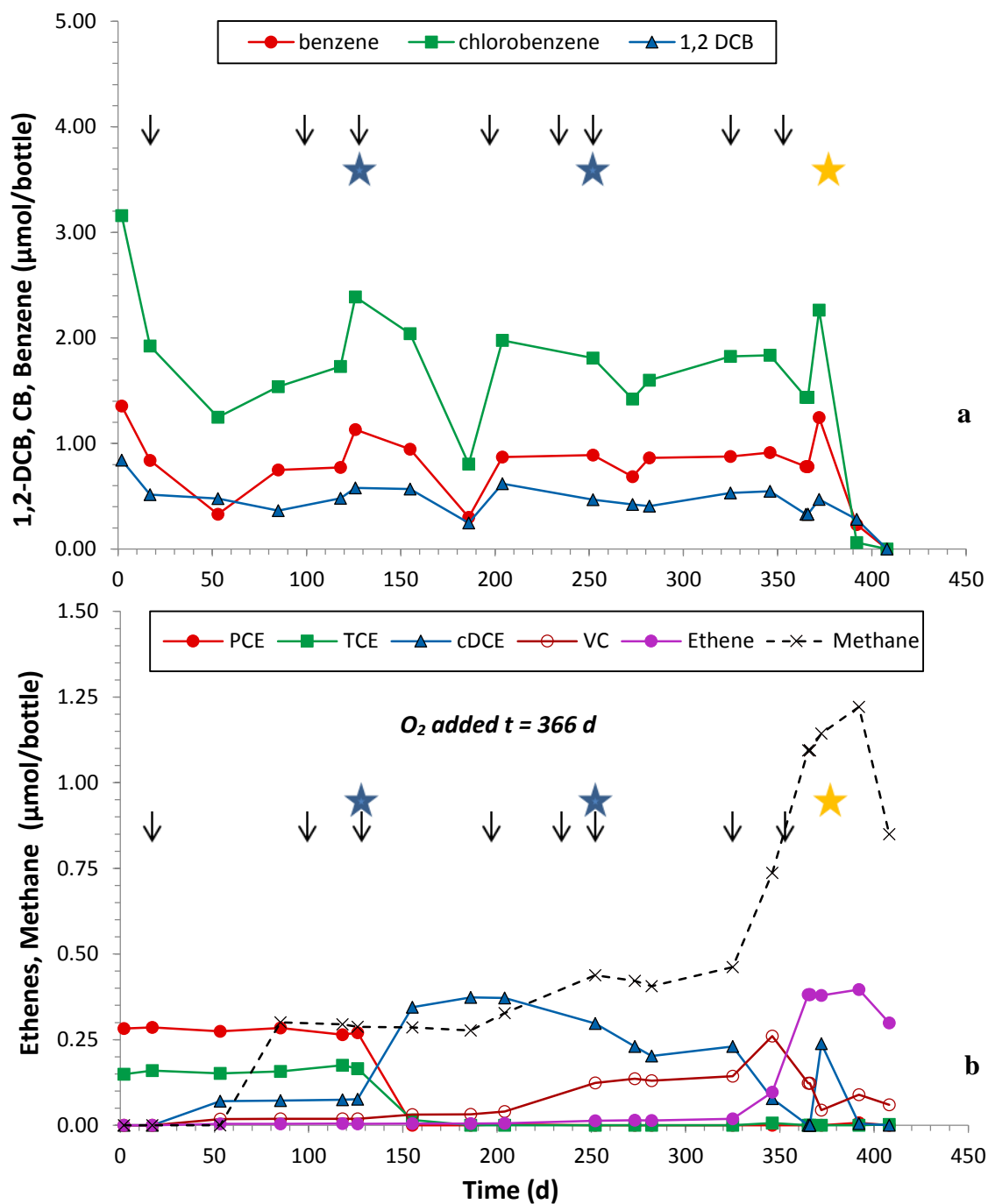


Figure 3.49. Results for downgradient plume bioaugmented anaerobic/aerobic microcosm #1; a) aromatic compounds at high concentrations; and b) ethenes, ethane, and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture and yellow stars indicate addition of soil and groundwater from the WWTP-AER microcosms.

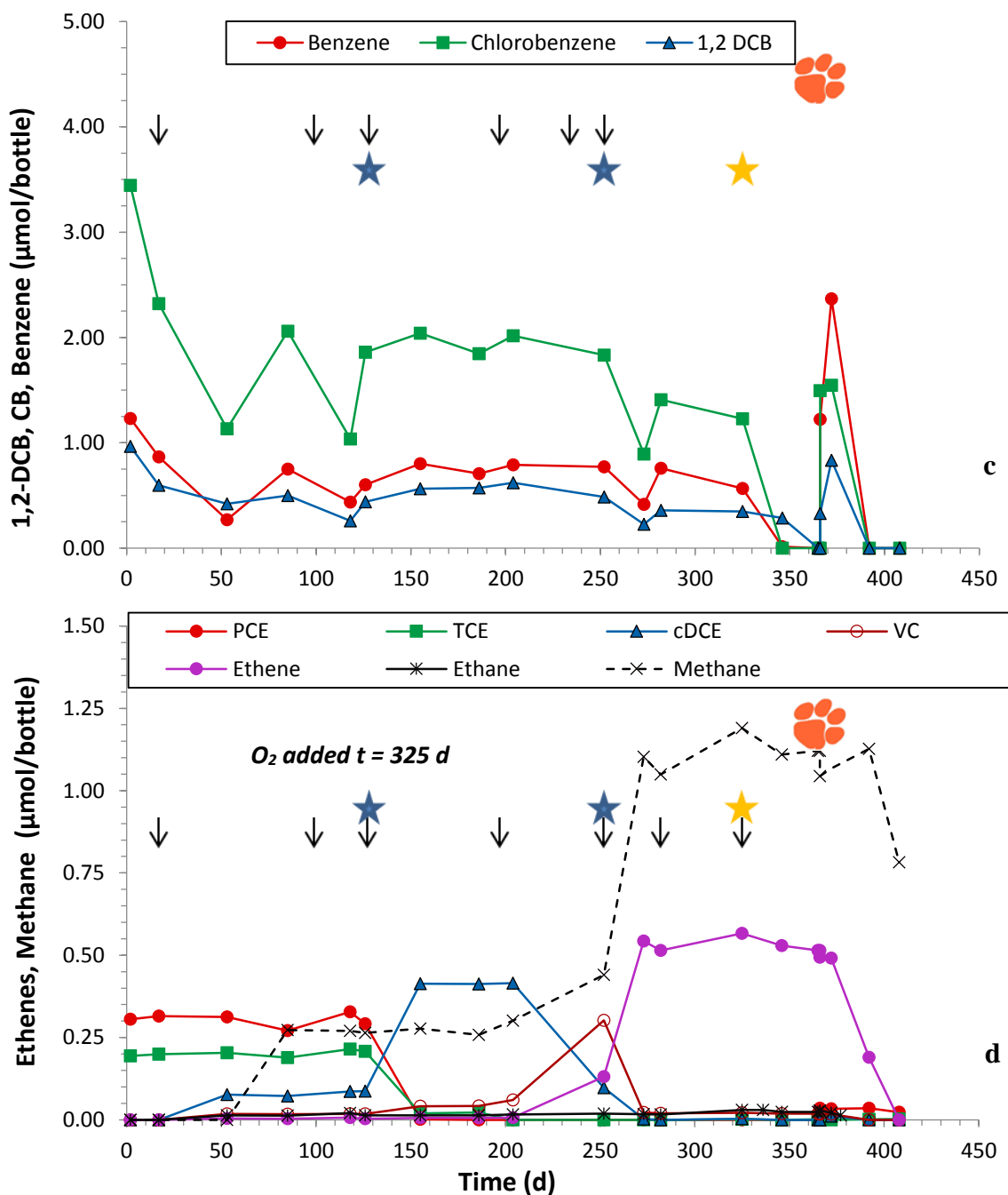


Figure 3.49 (continued). Results for downgradient plume bioaugmented anaerobic/aerobic microcosm #2; c) aromatic compounds at high concentrations; and d) ethenes, ethane, and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture, yellow stars indicate addition of soil and groundwater from the WWTP-AER microcosms, and tiger paws indicate re-addition of aromatic compounds.

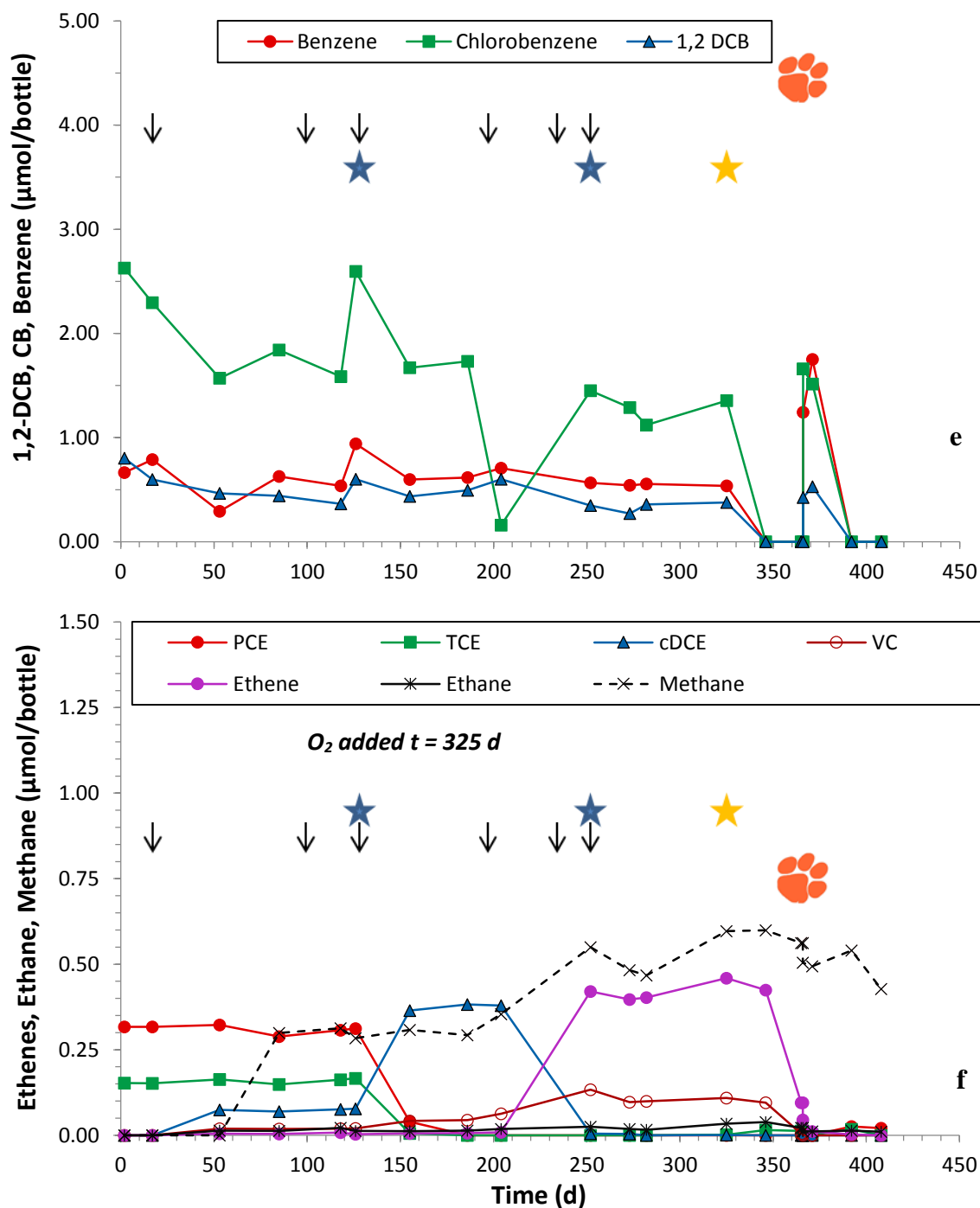


Figure 3.49 (continued). Results for downgradient plume bioaugmented anaerobic/aerobic microcosm #2; e) aromatic compounds at high concentrations; and f) ethenes, ethane, and methane. Arrows indicate addition of lactate; blue stars indicate addition of the KB-1 culture, yellow stars indicate addition of soil and groundwater from the WWTP-AER microcosms, and tiger paws indicate re-addition of aromatic compounds.

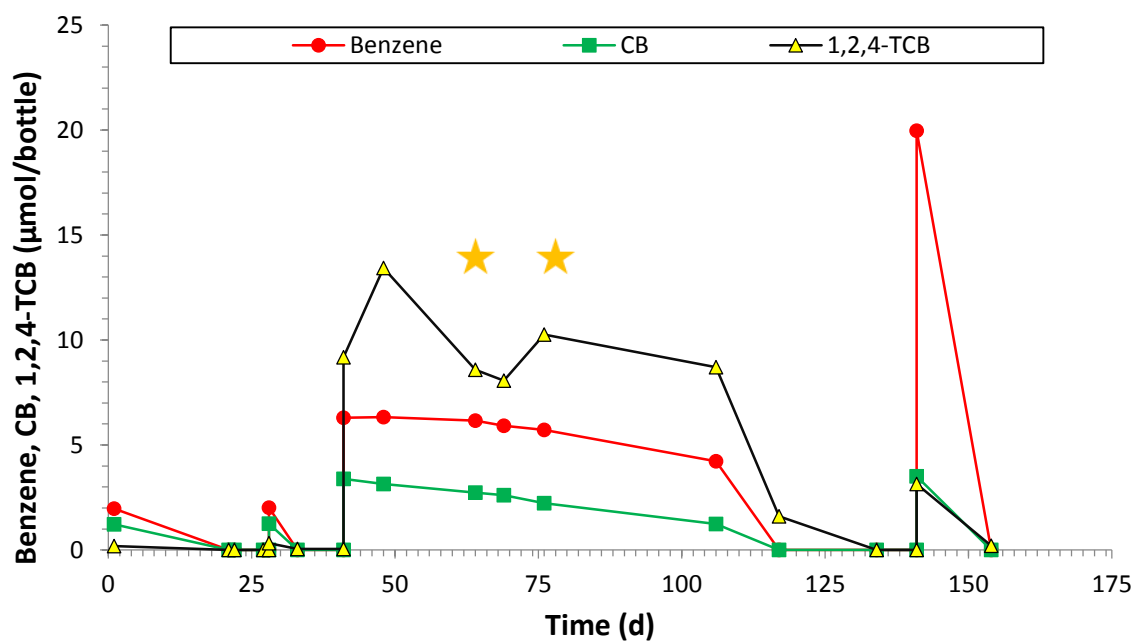


Figure 3.50 Results for the aerobic enrichment culture. Average of triplicate bottles. Stars indicate addition of 1 mL soil from WWTP aerobic microcosms.

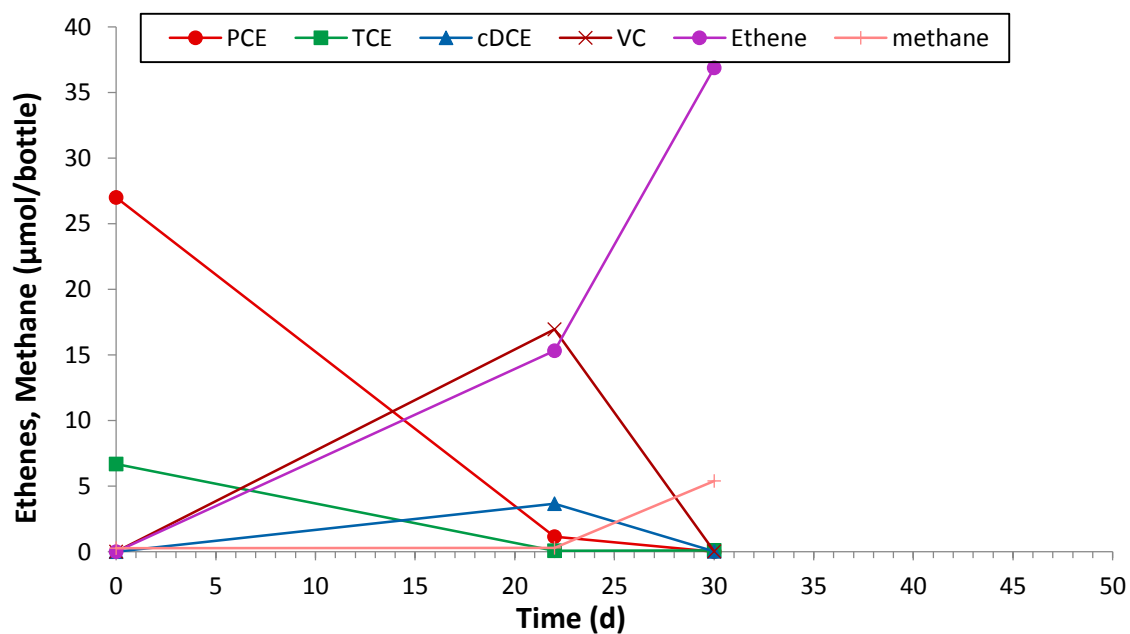


Figure 3.51 Results for the inhibition test containing chlorinated ethenes only. Averages of triplicate bottles.

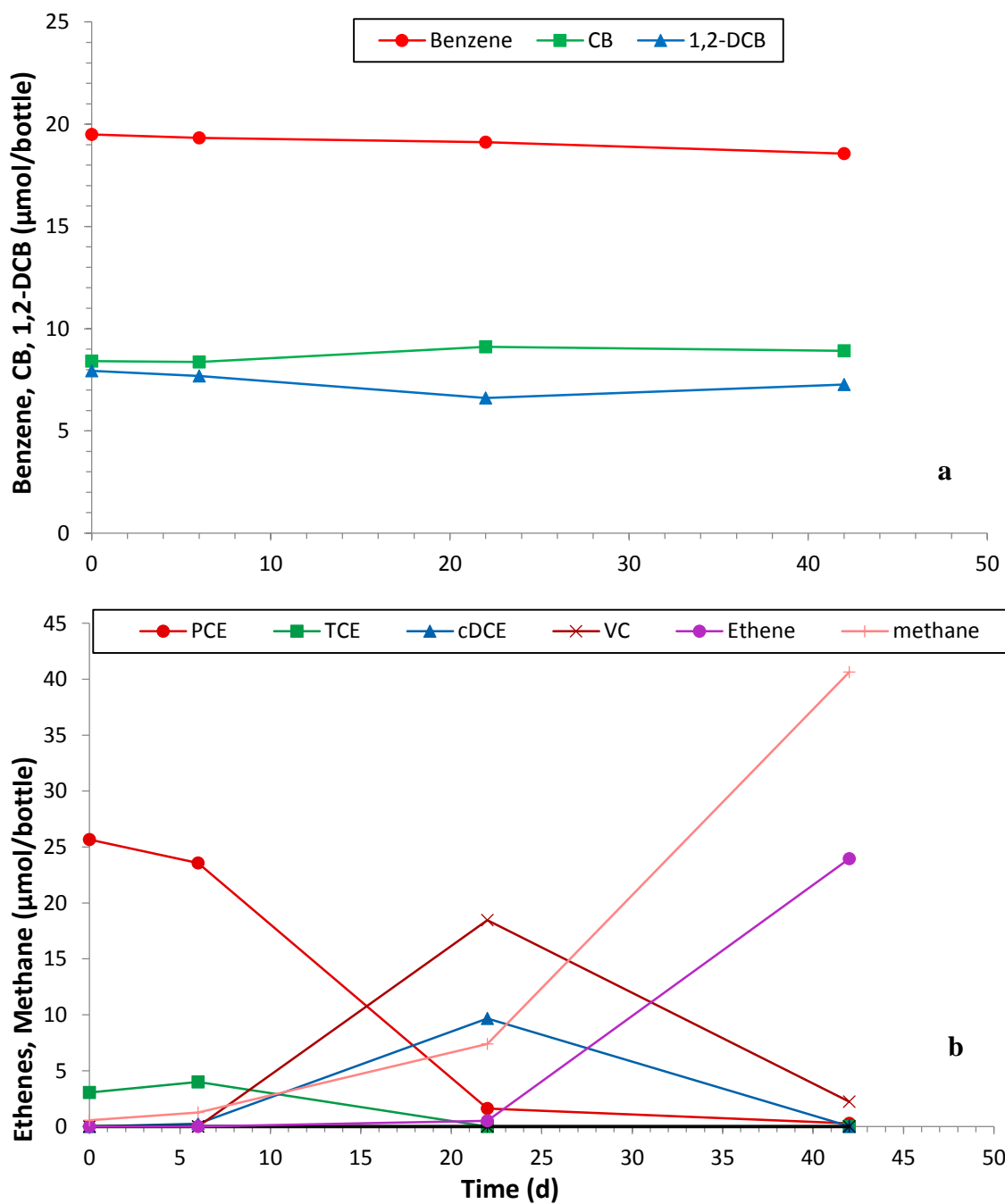


Figure 3.52 Results for the inhibition test containing aromatics at tank farm levels. Graphs show averages of triplicates. a) Concentration of aromatics present, b) concentrations of chlorinated ethenes, ethene and methane. Arrows indicate an addition of lactate.

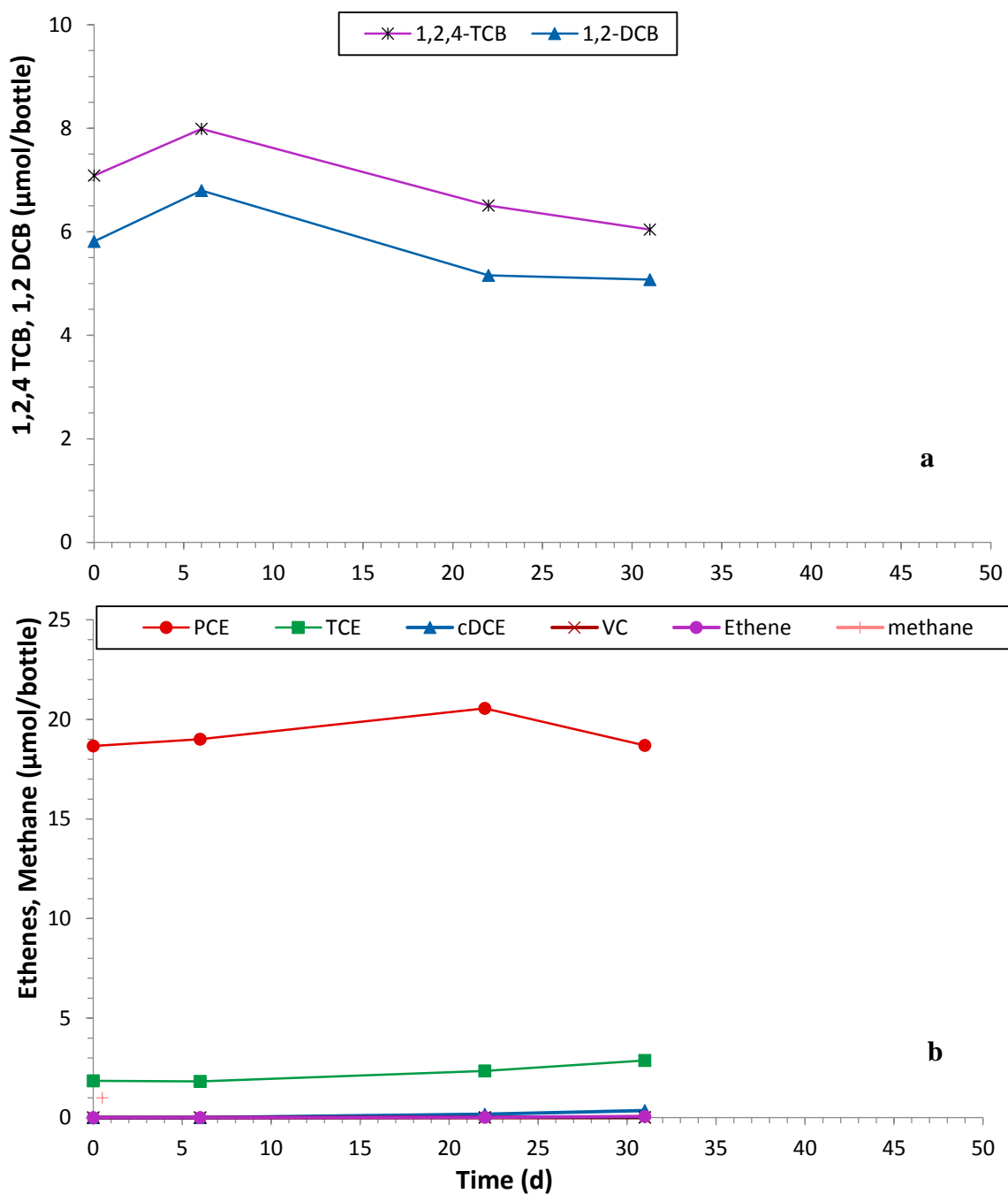


Figure 3.53 Results for the inhibition test containing aromatics at grease trap levels. Graphs show averages of triplicates. a) Concentration of aromatics present, b) concentrations of chlorinated ethenes, ethene and methane. Arrows indicate an addition of lactate.

APPENDICES

Appendix A: Media Preparation

Anaerobic MSM

Stock solutions prepared for medium preparation:

Phosphate buffer

In a 100 mL volumetric flask add 5.25 g K_2HPO_4 . Fill to 100 mL with DDI water.

- Salt solution

In a 100 mL volumetric flask add:

5.35 g NH_4Cl

0.46976 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

0.17787 g $\text{FeCl}_2 \cdot \text{H}_2\text{O}$

Fill to 100 mL with DDI water.

- Trace metals solution

In a 100 mL volumetric flask add:

0.03 g H_3BO_3

0.0211 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$

0.075 g $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$

0.1 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$

0.01 g $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$

0.15 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$

0.002 g Na_2SeO_3

0.01 g $\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$

1 mL HCl, 37%.

Fill to 100 mL with DDI water.

- Magnesium sulfate solution

In a 100 mL volumetric flask add 6.25 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Fill to 100 mL with DDI water.

- Redox solution

In a 10 mL volumetric flask add 0.01 g resazurin. Fill to 10 mL with DDI water.

- Bicarbonate solution

In a 500 mL volumetric flask add 8 g NaHCO_3 . Fill to 500 mL with DDI water.

- Yeast extract solution

In a 100 mL volumetric flask add 0.5 g yeast extract. Fill to 100 mL with DDI water.

- Ferrous sulfide

For 1 L of media, weigh into separate glass vials:

0.24 g of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$

0.1448 g $\text{FeCl}_2 \cdot \text{H}_2\text{O}$

Medium was then prepared with the following steps.

1) In a 1 L bottle add:

10 mL phosphate solution

10 mL salt solution

2 mL trace metals solution

2 mL magnesium sulfate solution

1 mL redox solution

965 mL DDI water

2) Autoclave the above solution and allow to cool.

- 3) Add: 10 mL filter sterilized yeast extract
- 4) Transfer the bottle to the anaerobic chamber along with the vials of sodium sulfide and ferrous chloride and 10 mL of sterile DDI water. When the O_2 reaches zero, add the 0.24 g of $Na_2S \cdot 9H_2O$ and rinse the vial with ~5 mL of sterile DDI water. Wait until the media turns from pink to clear.
- 5) Add the 0.1448 g $FeCl_2 \cdot H_2O$. Rinse the vial with ~5 mL of sterile DDI water.

Aerobic MSM

Prepared in a 1L volumetric flask:

MgSO₄· 7H₂O, 112 mg

ZnSO₄· 7H₂O, 5 mg

Na₂MoO₄· 2H₂O, 2.5 mg

KH₂PO₄, 340 mg

Na₂HPO₄ 7H₂O, 670 mg

CaCl₂, 14 mg; FeCl₃

0.13 mg; and NH₄Cl, 0.5 g

Distilled deionized water used to fill volumetric flask.

The pH is then adjusted to 7.0.

Appendix B: Response Factors

Table B.1. Oxygen response factors.

Date	Response Factor (% O ₂ /Peak Area Unit)
25-March-2014	2.9167
9-April-2014	3.1792
14-April-2014	3.0477
28-April-2014	3.0269
10-May-2014	3.2794
26-May-2014	3.3728
9-June-2014	3.4329
27-June-2014	3.4107
23-July-2014	3.0179
19-August-2014	3.0459
1-September-2014	2.9976
16-September-2014	2.9099
28-October-2014	3.1703
18-November-2014	3.0488
29-November-2014	3.1139
8-December-2014	2.6635
6-Janurary-2015	2.5627
19-Janurary-2015	2.8517
2-February-2015	2.8046
12-February-2015	3.1527
25-February-2015	2.6661
15-March-2015	2.7612
20-April-2015	4.6143

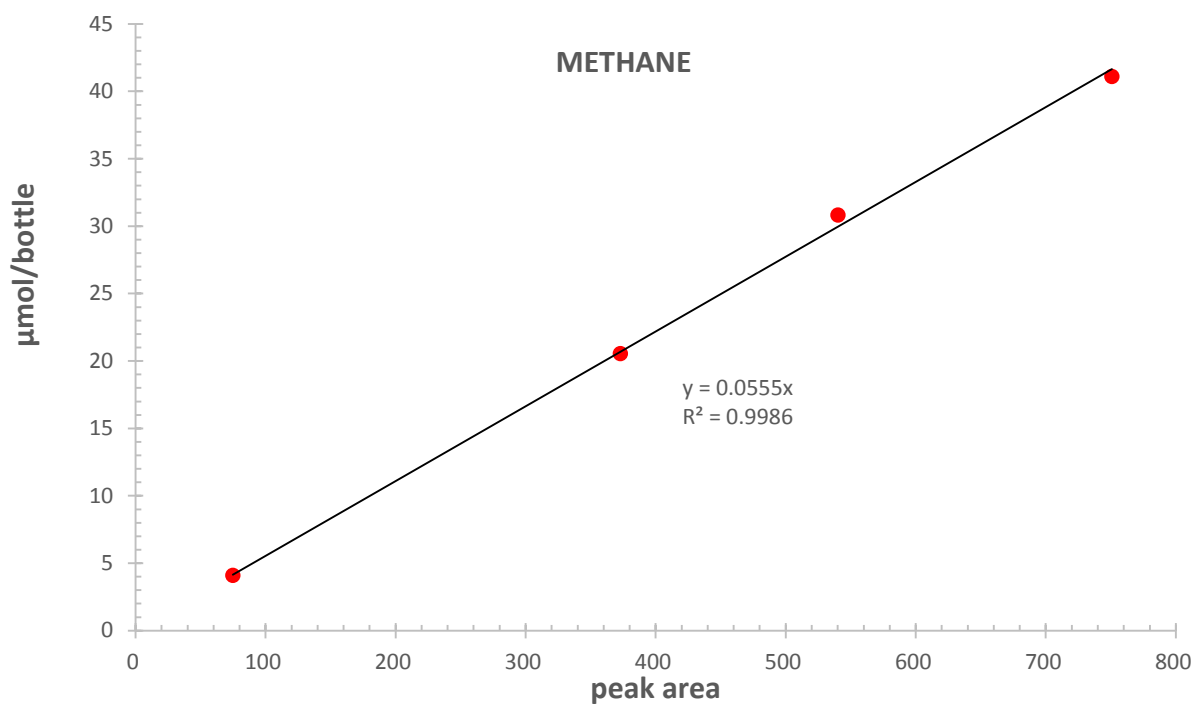


Figure B.1. Calibration curve for methane for the HP 5890 Series II GC.

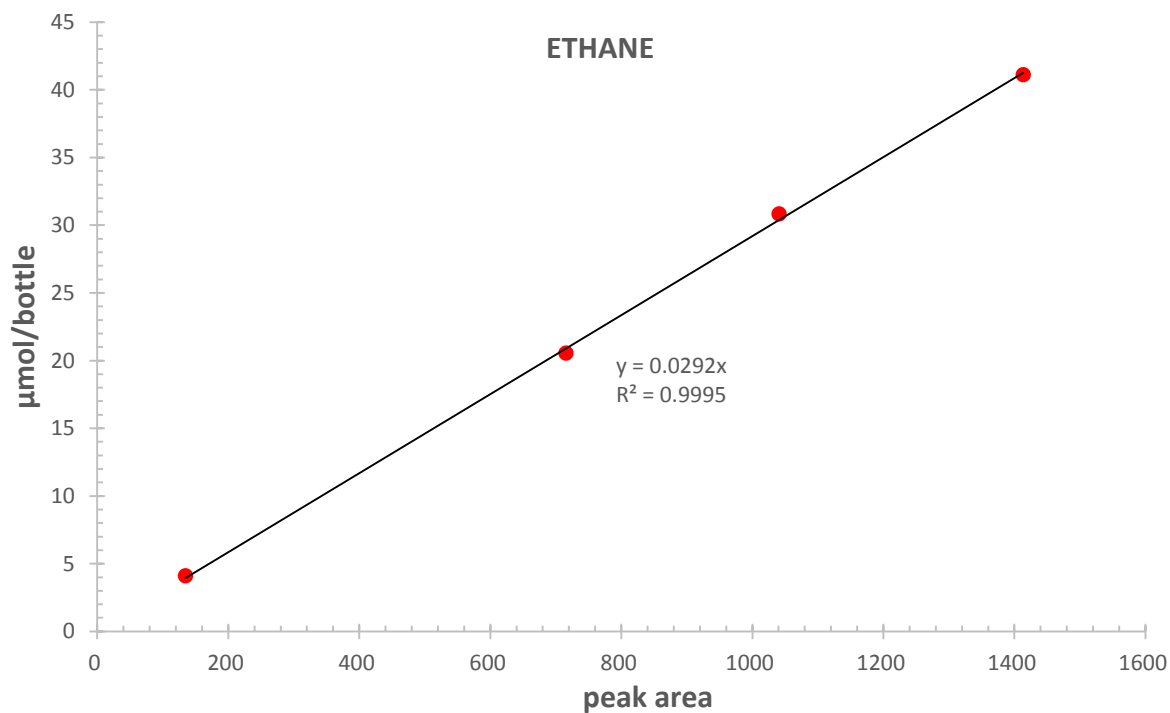


Figure B.2. Calibration curve for ethane for the HP 5890 Series II GC.

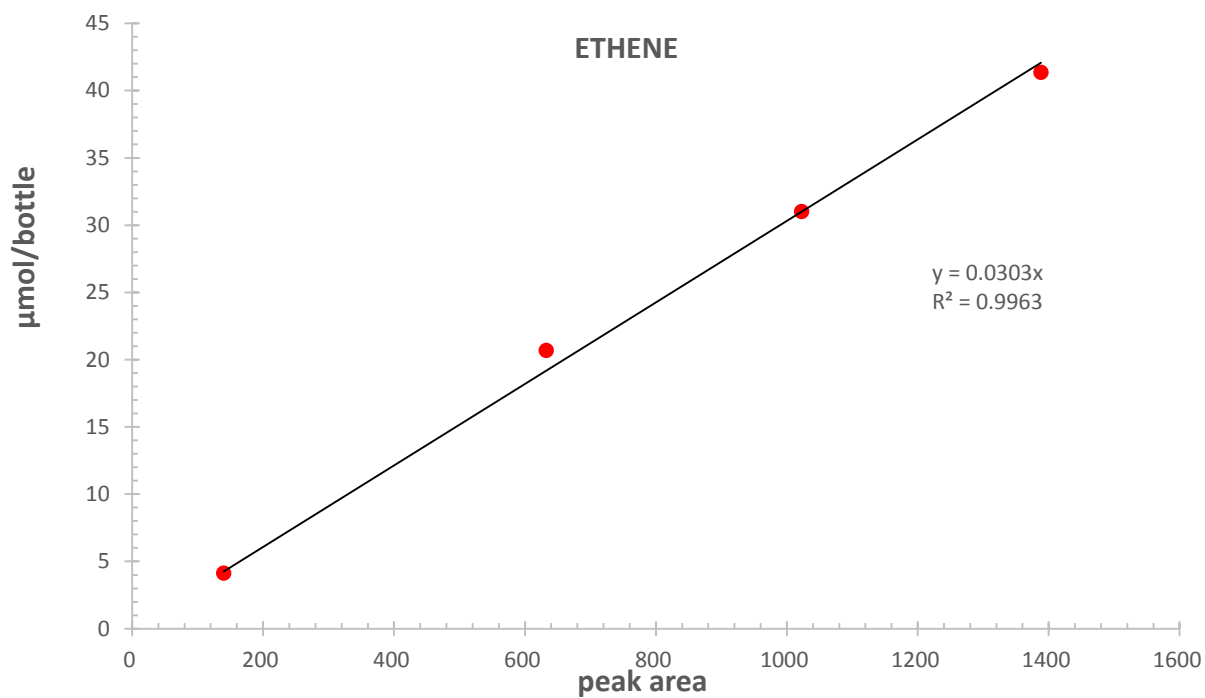


Figure B.3. Calibration curve for ethene for the HP 5890 Series II GC.

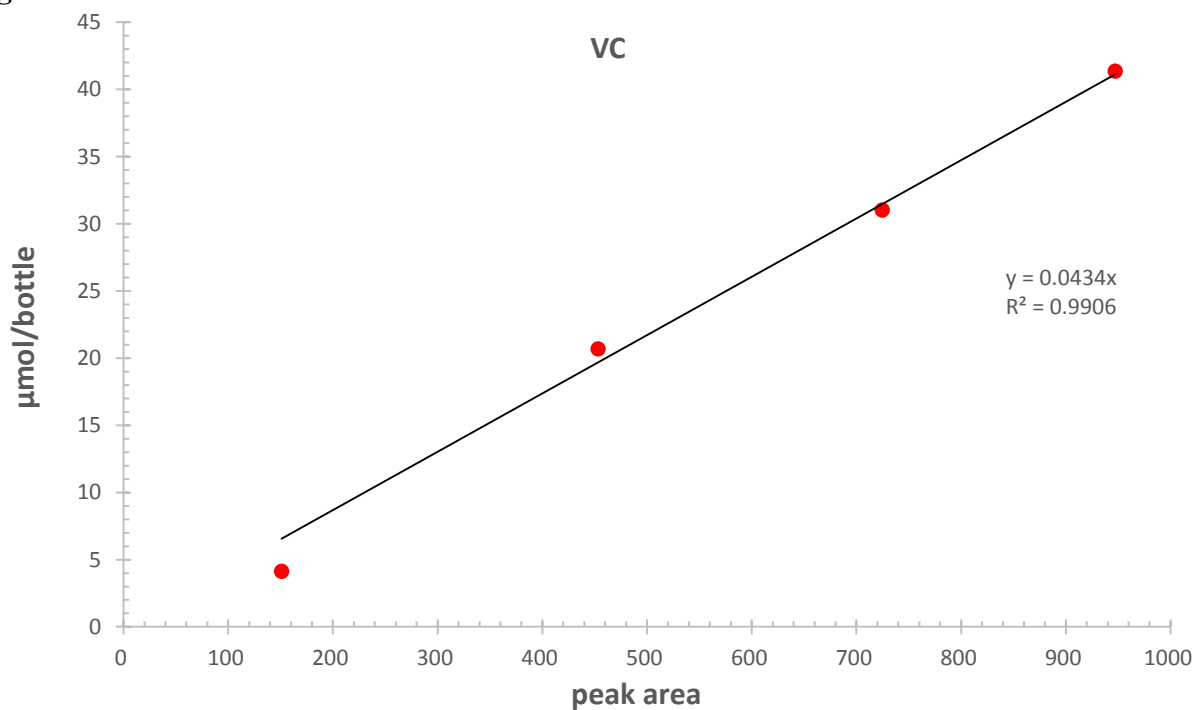


Figure B.4. Calibration curve for vinyl chloride for the HP 5890 Series II GC.

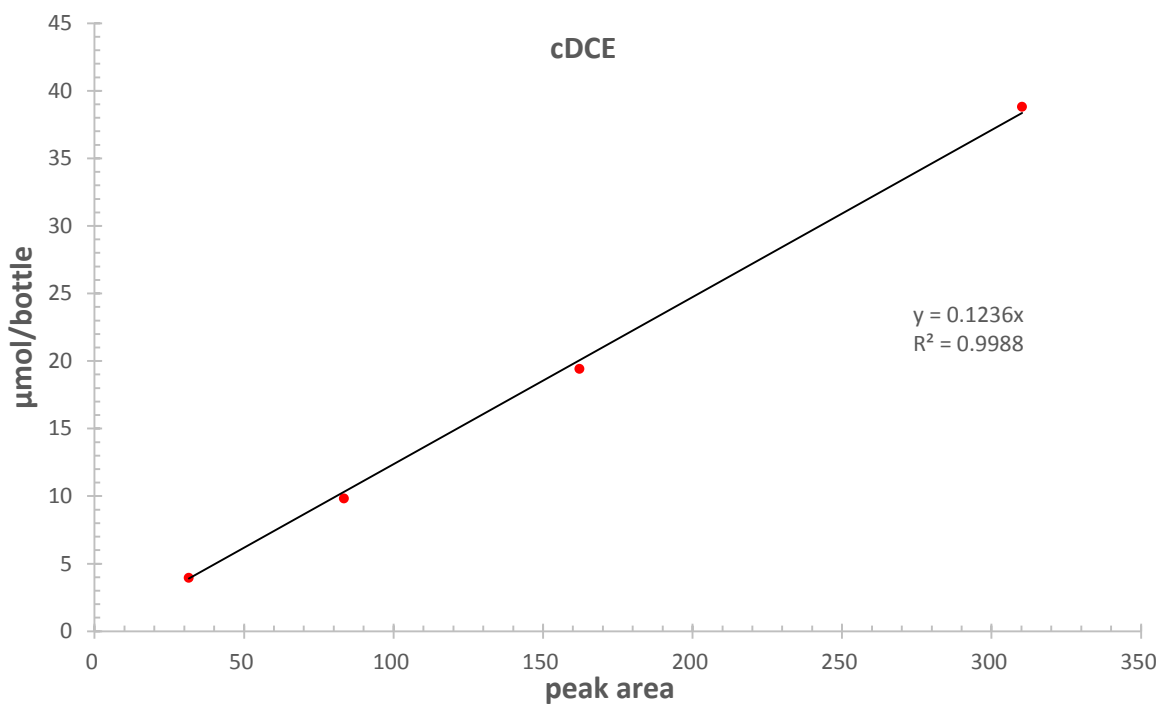


Figure B.5. Calibration curve for cDCE for the HP 5890 Series II GC.

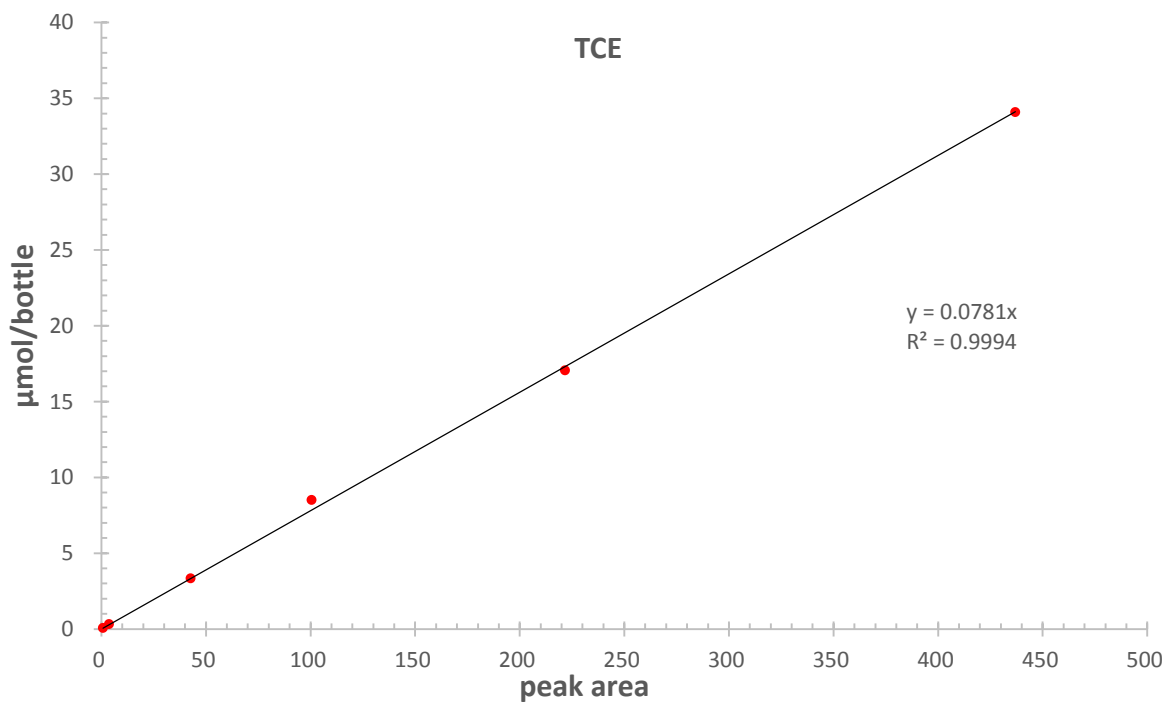


Figure B.6. Calibration curve for TCE for the HP 5890 Series II GC.

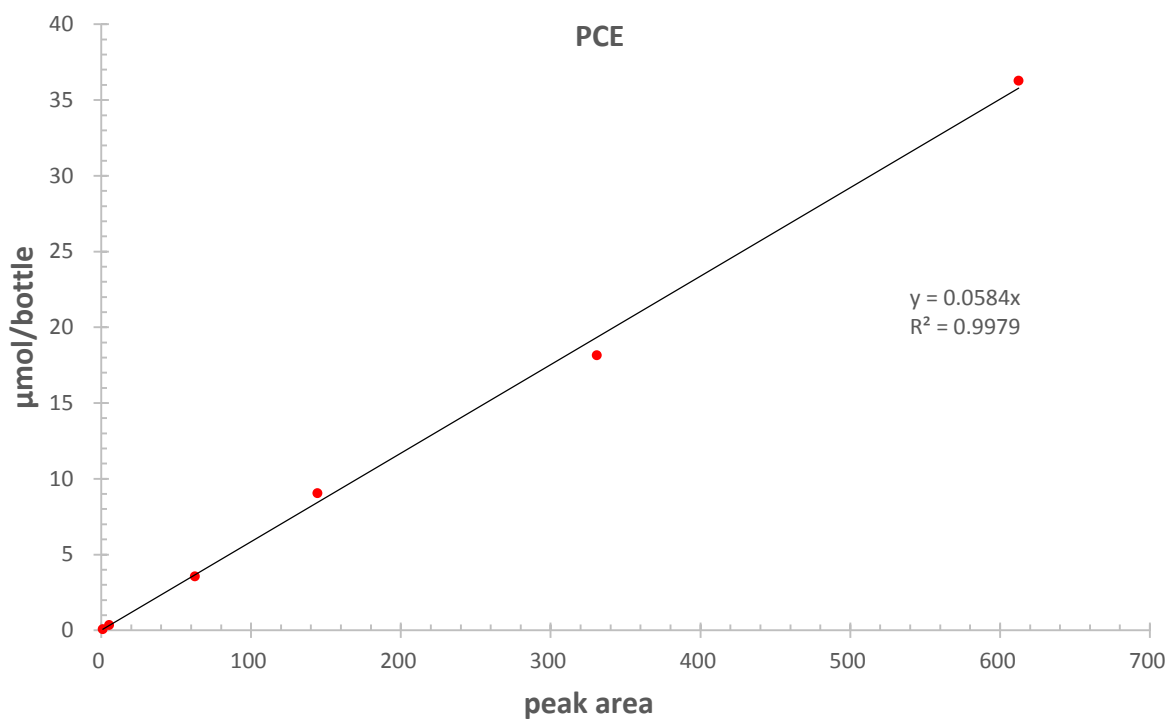


Figure B.7. Calibration curve for PCE for the HP 5890 Series II GC.

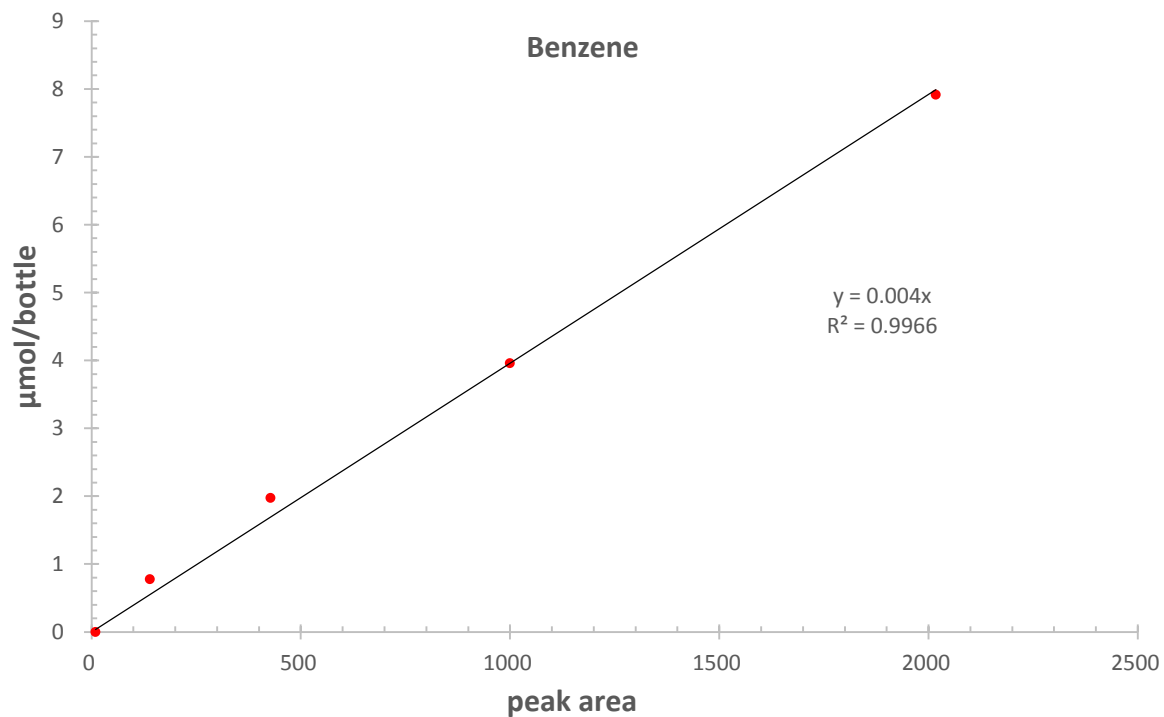


Figure B.8. Calibration curve for benzene for the HP 6890 GC.

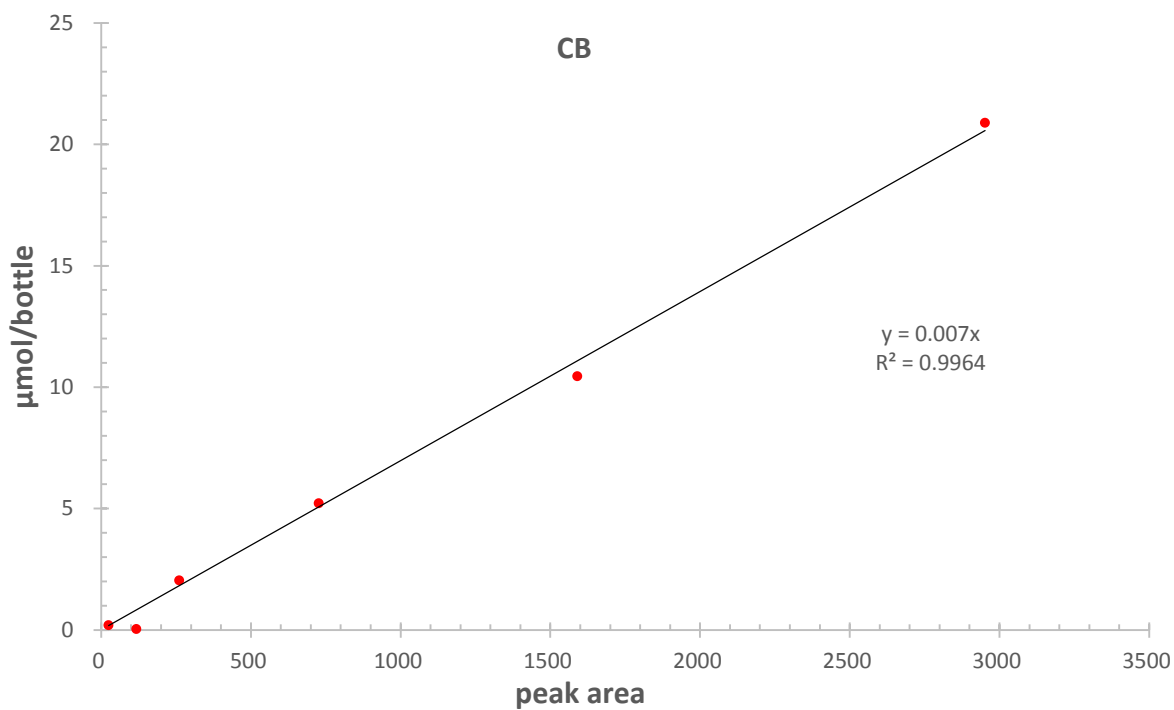


Figure B.9. Calibration curve for chlorobenzene for the HP 6890 GC.

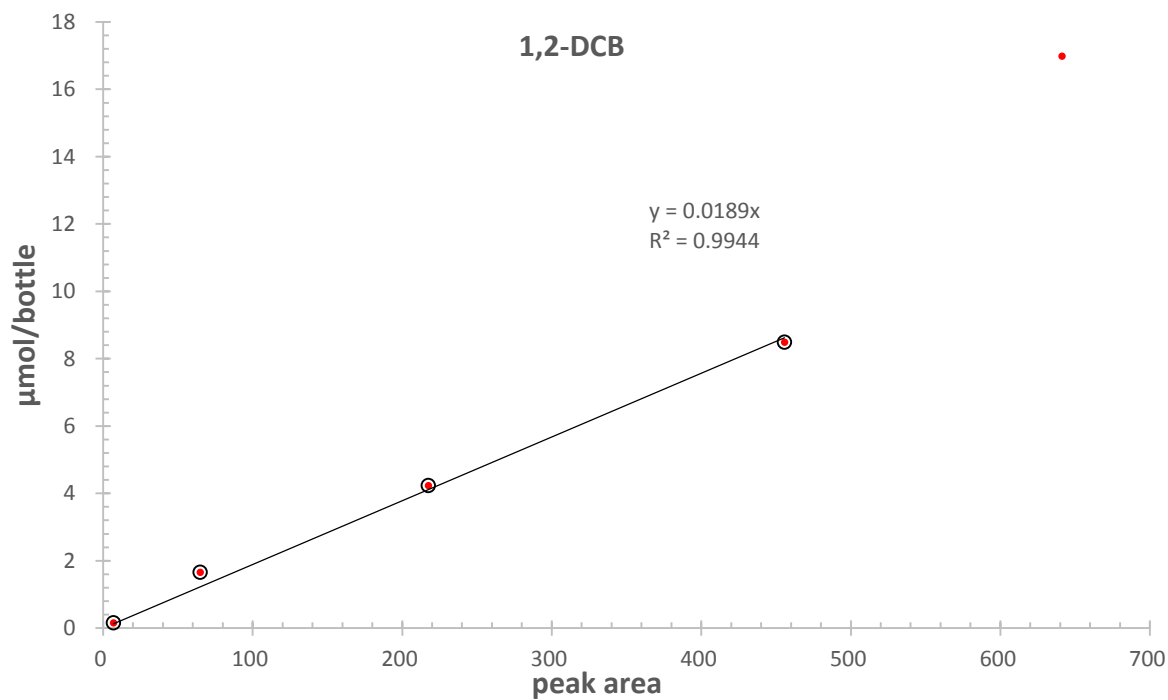


Figure B.10. Calibration curve for 1,2 DCB for the HP 6890 GC.

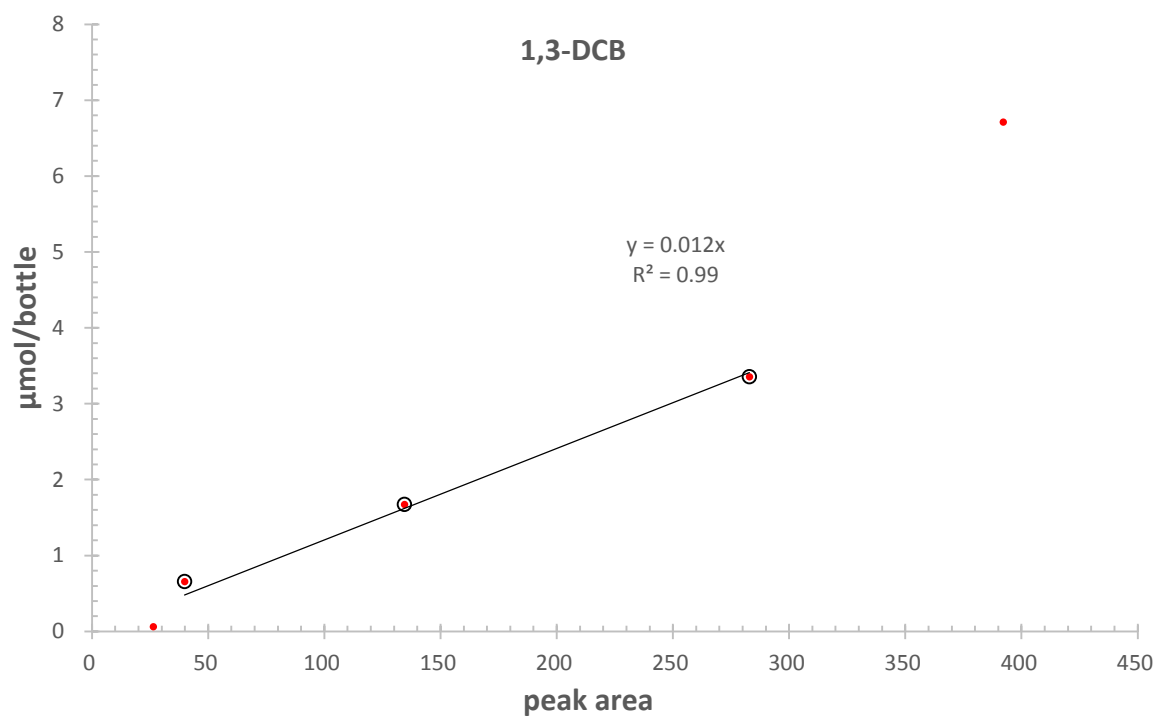


Figure B.11. Calibration curve for 1,3 DCB for the HP 6890 GC.

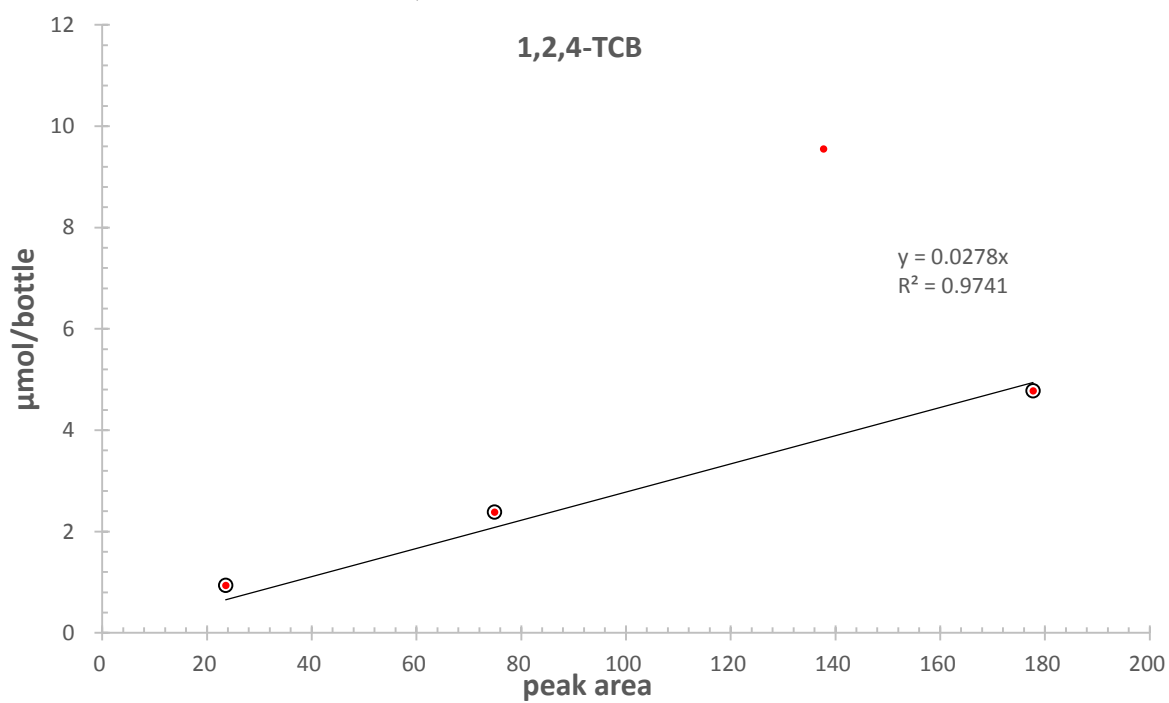


Figure B.12. Calibration curve for 1,2,4 TCB for the HP 6890 GC.

Appendix C: Low Concentration Aromatic Graphs

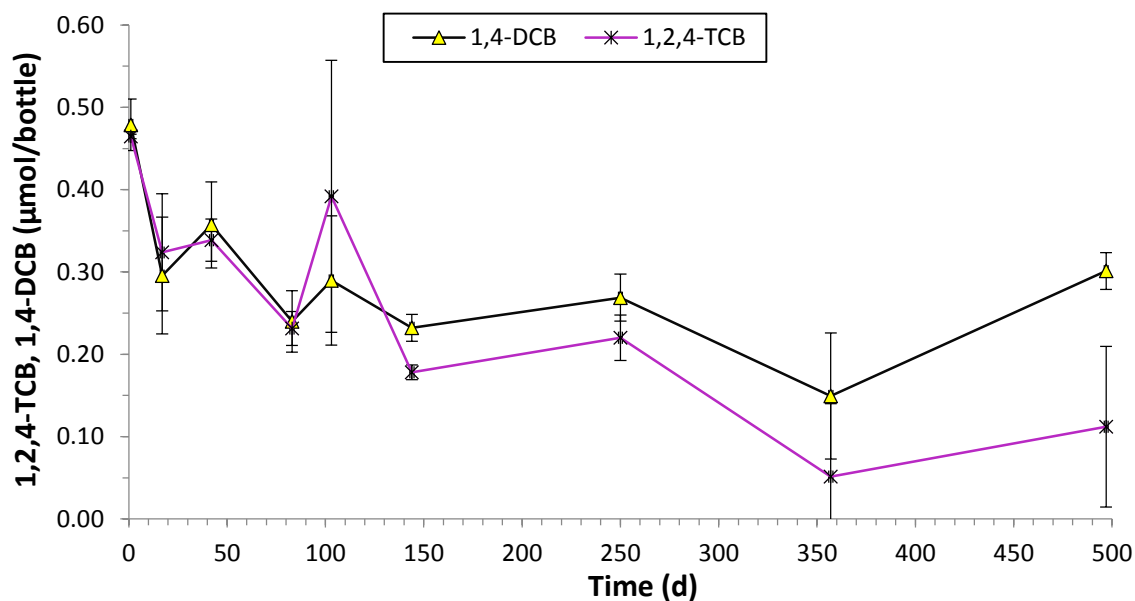


Figure C.1. Results for the triplicate WWTP autoclaved control microcosms; low concentration chlorinated benzenes. Error bars represent standard deviations.

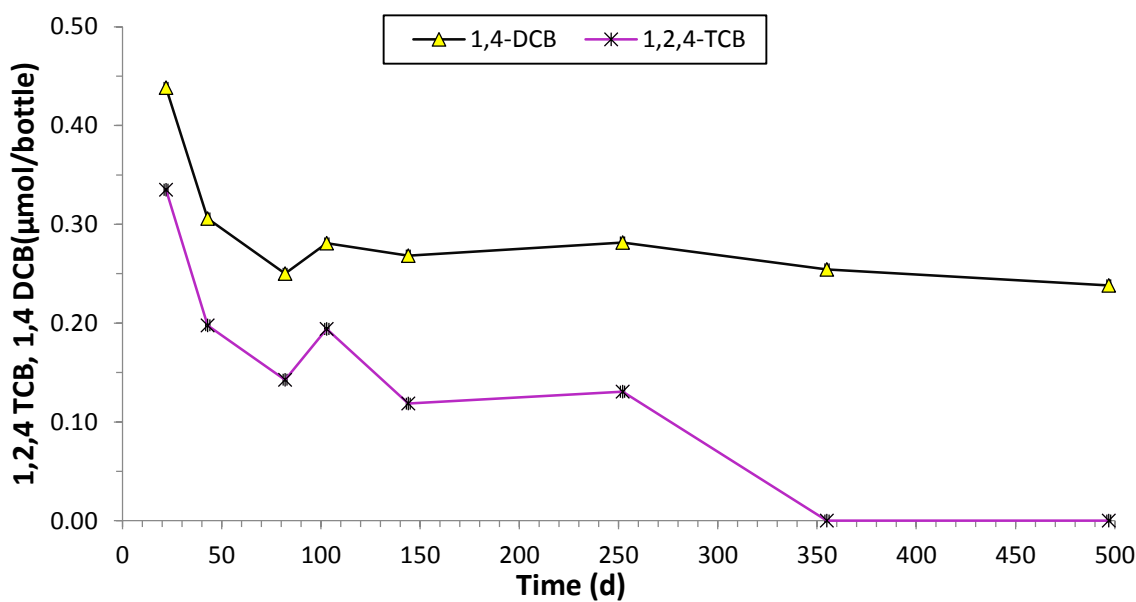


Figure C.2. Results for the triplicate WWTP unamended anaerobic microcosms; low concentration chlorinated benzenes.

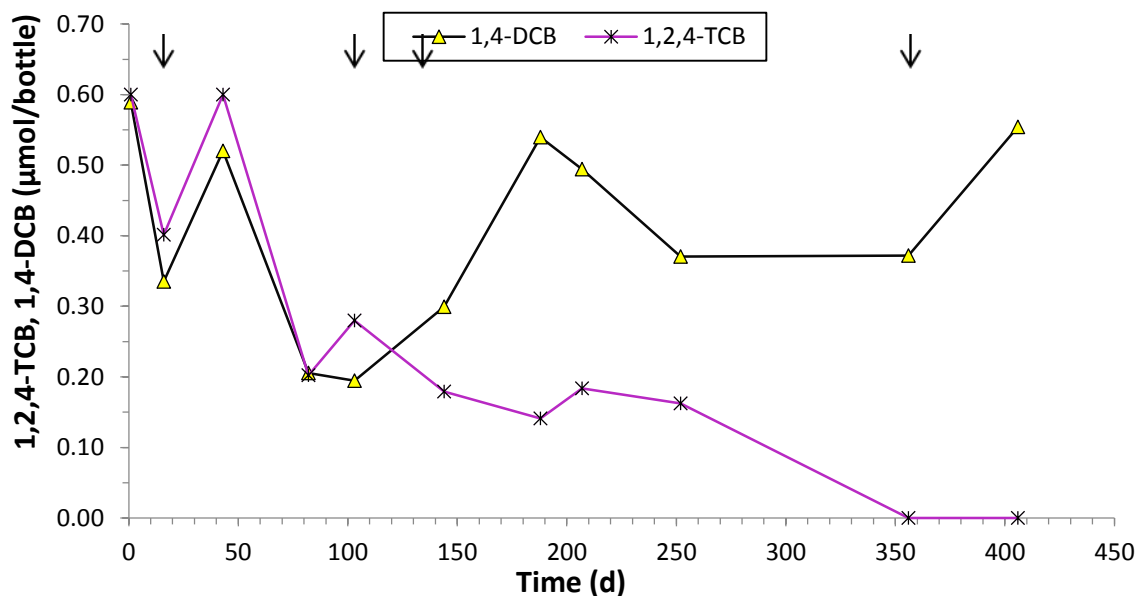


Figure C.3. Results for the triplicate WWTP anaerobic lactate-amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

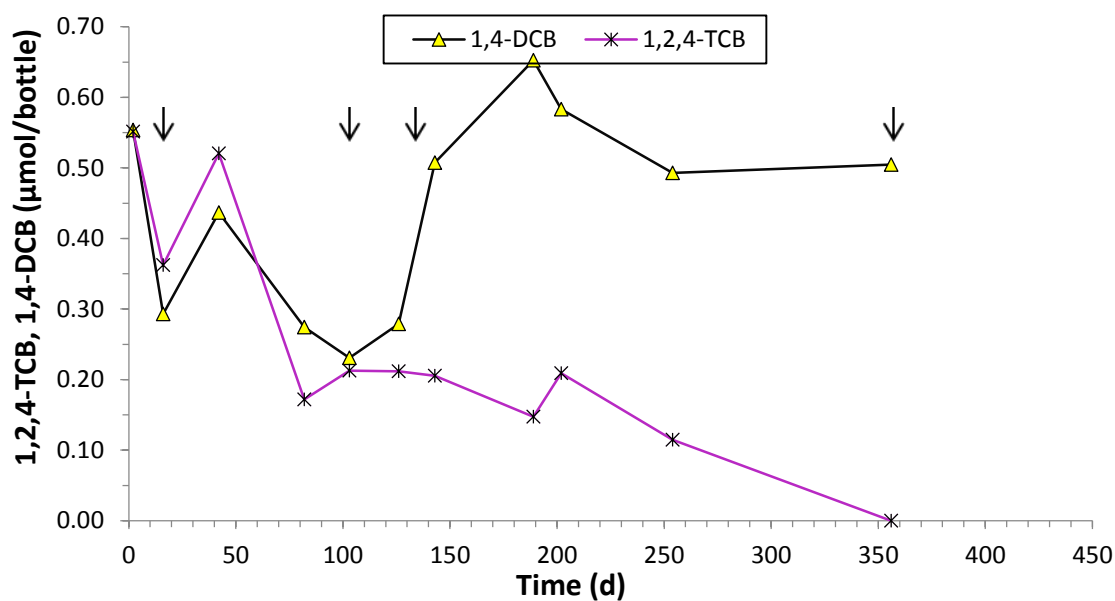


Figure C.4. Results for the triplicate WWTP EVO-amended anaerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of EVO.

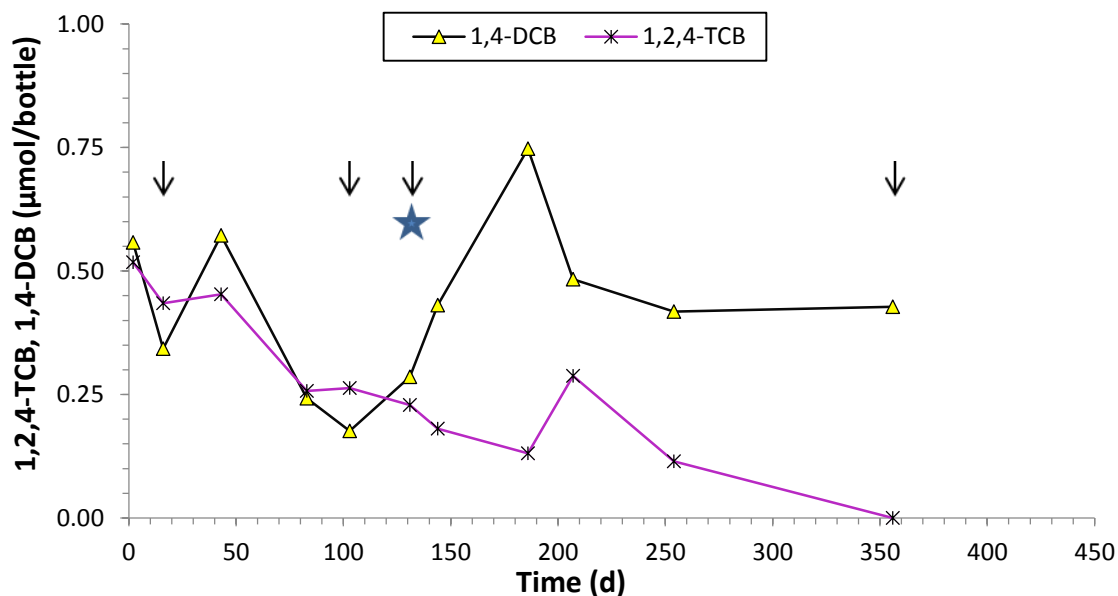


Figure C.5. Results for the triplicate WWTP bioaugmented anaerobic microcosms. low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of KB-1 bioaugmentation culture.

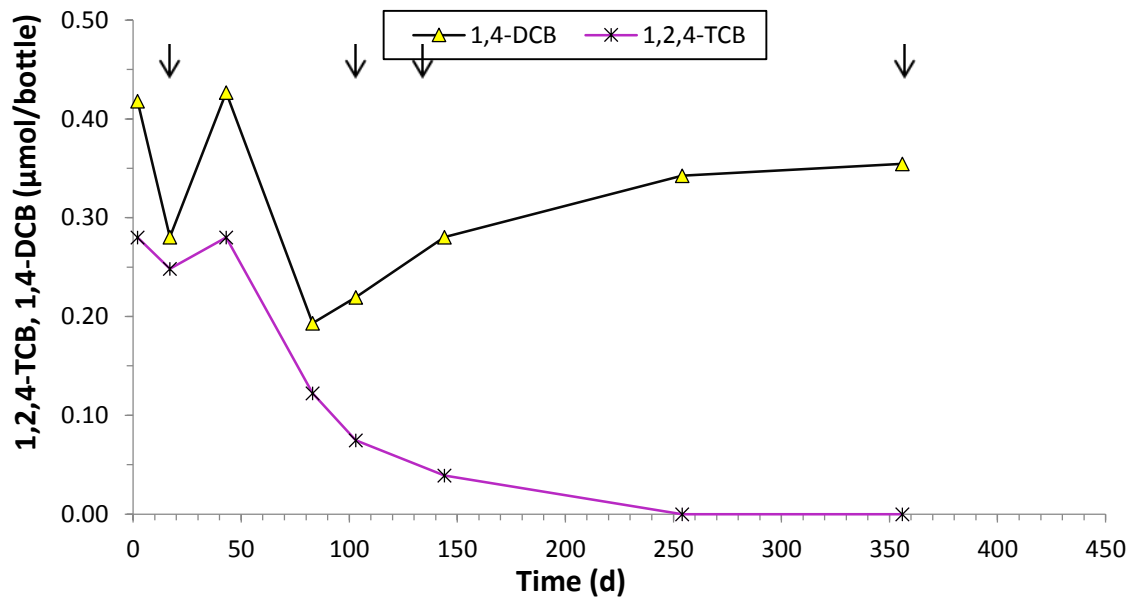


Figure C.6. Results for the triplicate WWTP ZVI and lactate amended anaerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

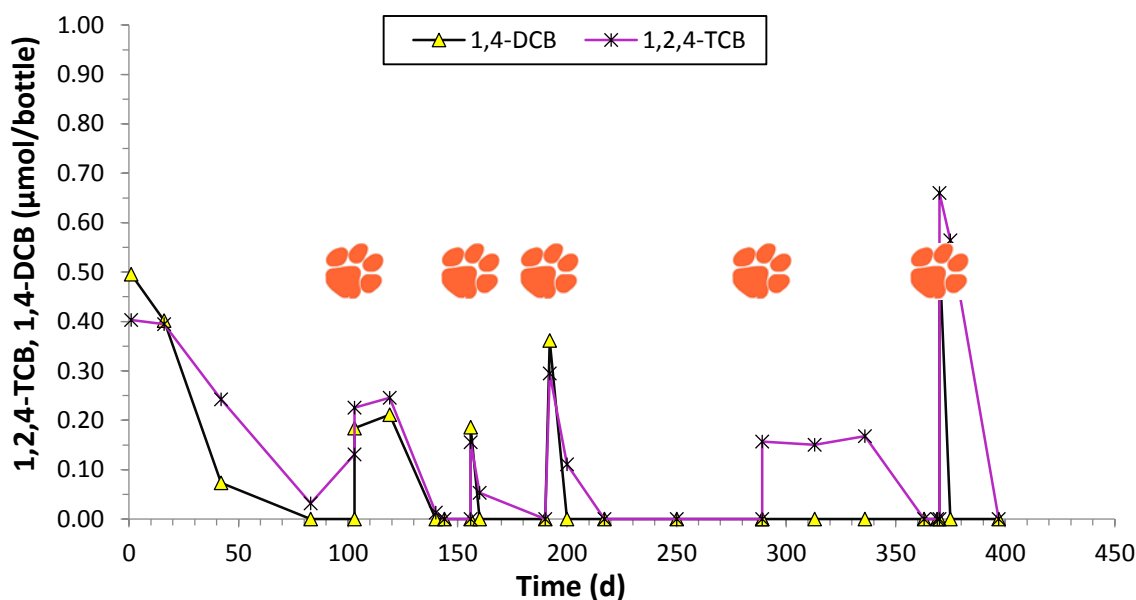


Figure C.7. Results for the triplicate WWTP unamended aerobic microcosms; low concentration chlorinated benzenes. Tiger paws indicate re-addition of chlorinated benzenes.

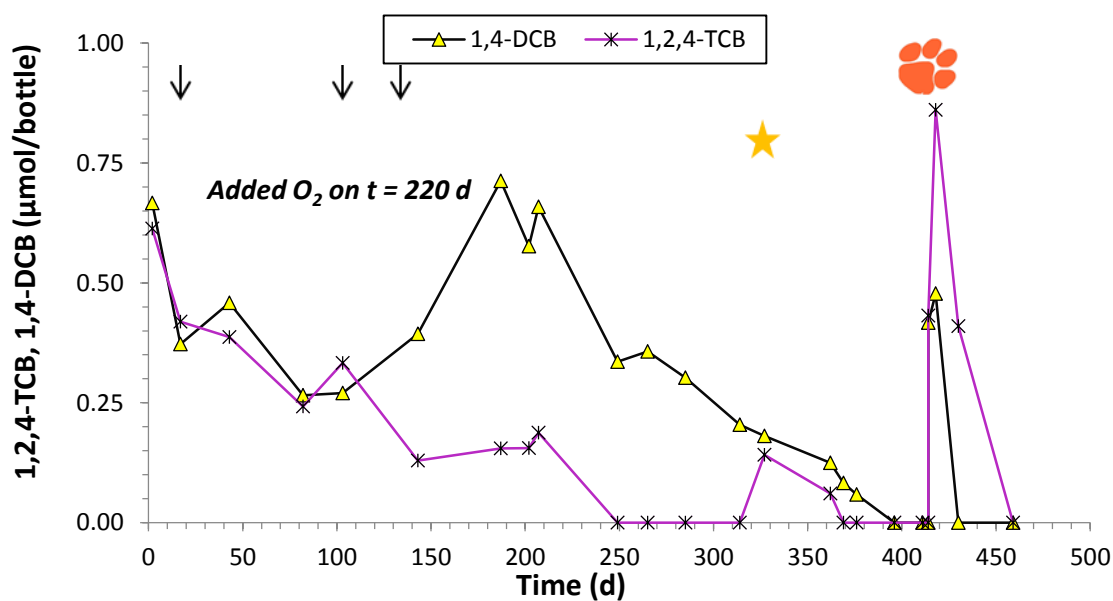


Figure C.8. Results for the triplicate WWTP lactate-amended anaerobic/aerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of soil from WWTP-AER microcosms. Tiger paws indicate re-addition of chlorinated benzenes.

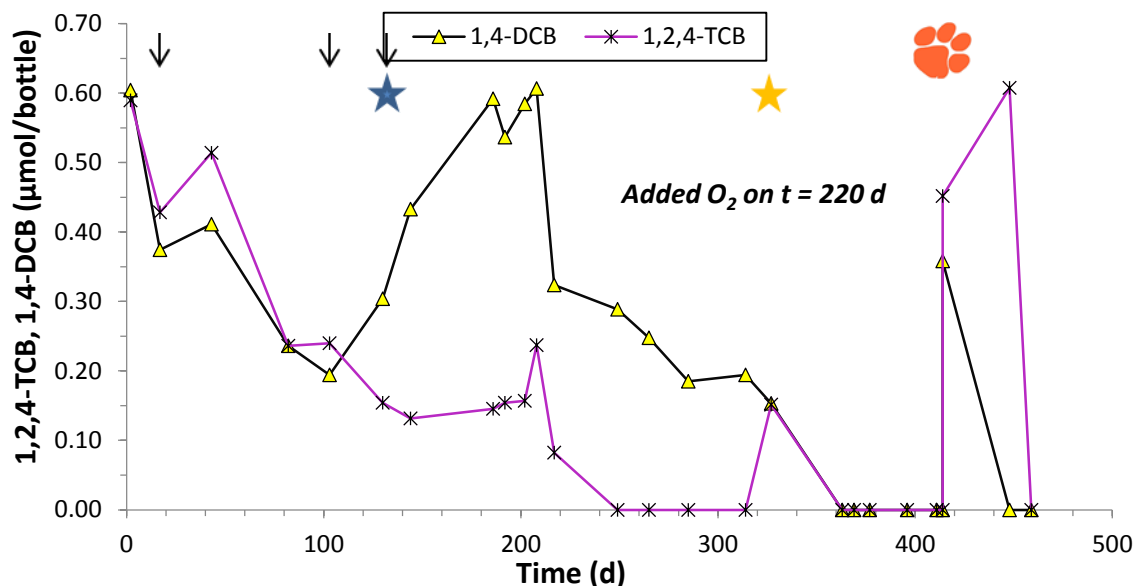


Figure C.9. Results for the triplicate WWTP bioaugmented anaerobic/aerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Blue stars indicate addition of KB-1 culture and yellow stars indicate addition of soil from the WWTP-AER microcosms. A Tiger paw indicates re-addition of chlorinated benzenes.

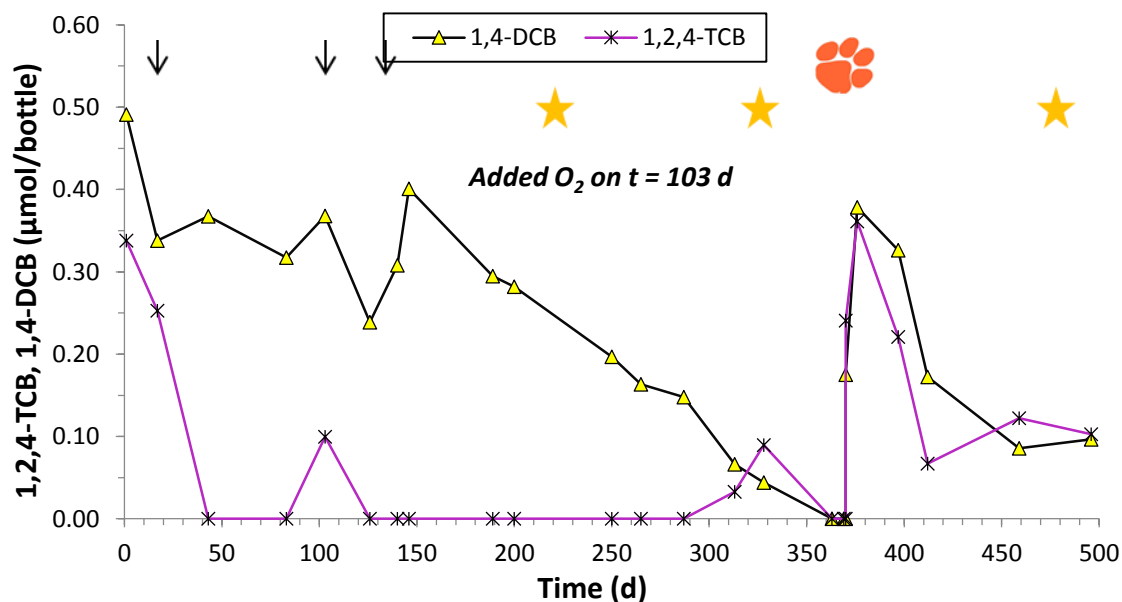


Figure C.10. Results for the triplicate WWTP ZVI and lactate-amended anaerobic/aerobic microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of soil from WWTP-AER microcosms. Tiger paws indicate re-addition of chlorinated benzenes.

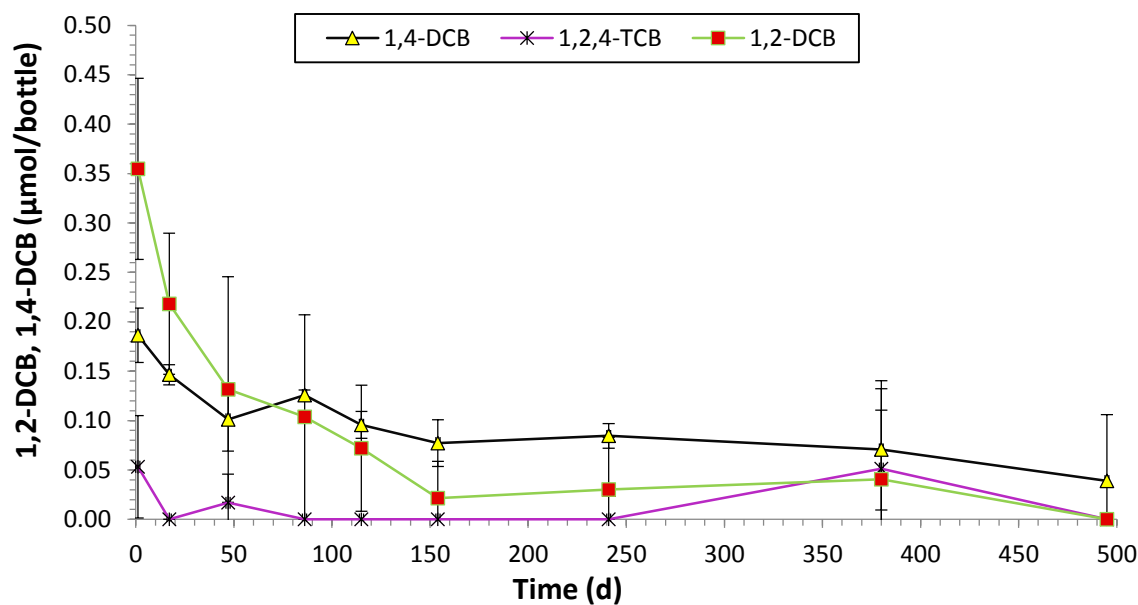


Figure C.11. Results for the triplicate ORP autoclaved microcosms: averages of triplicate bottles, Low concentrations chlorinated benzenes. Error bars represent standard deviations.

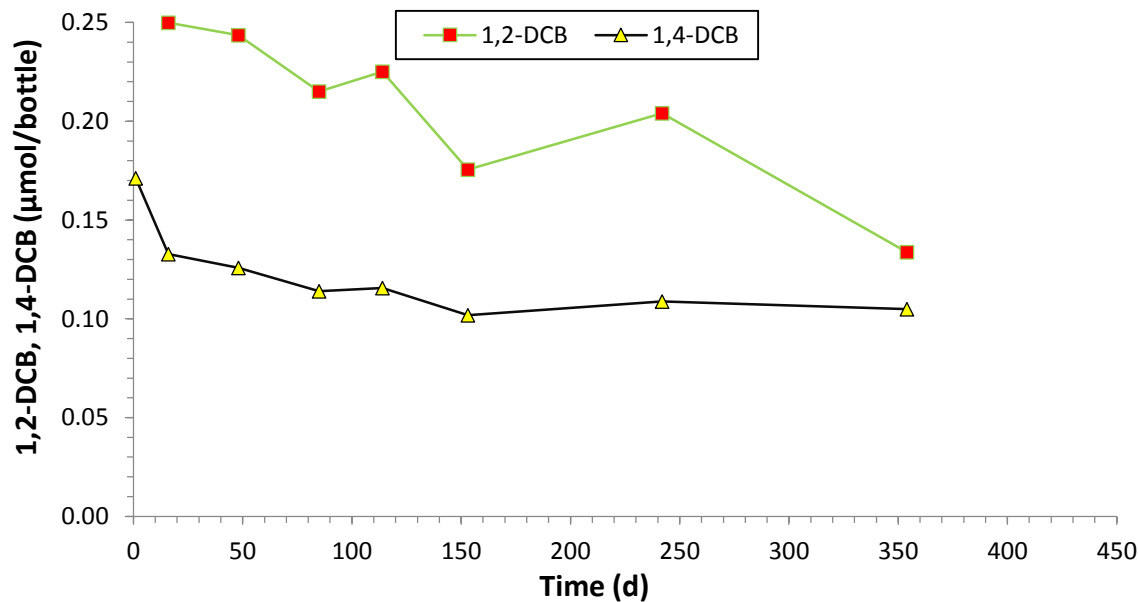


Figure C.12. Results for the triplicate ORP anaerobic unamended microcosms: averages of triplicate bottles, Low concentrations chlorinated benzenes.

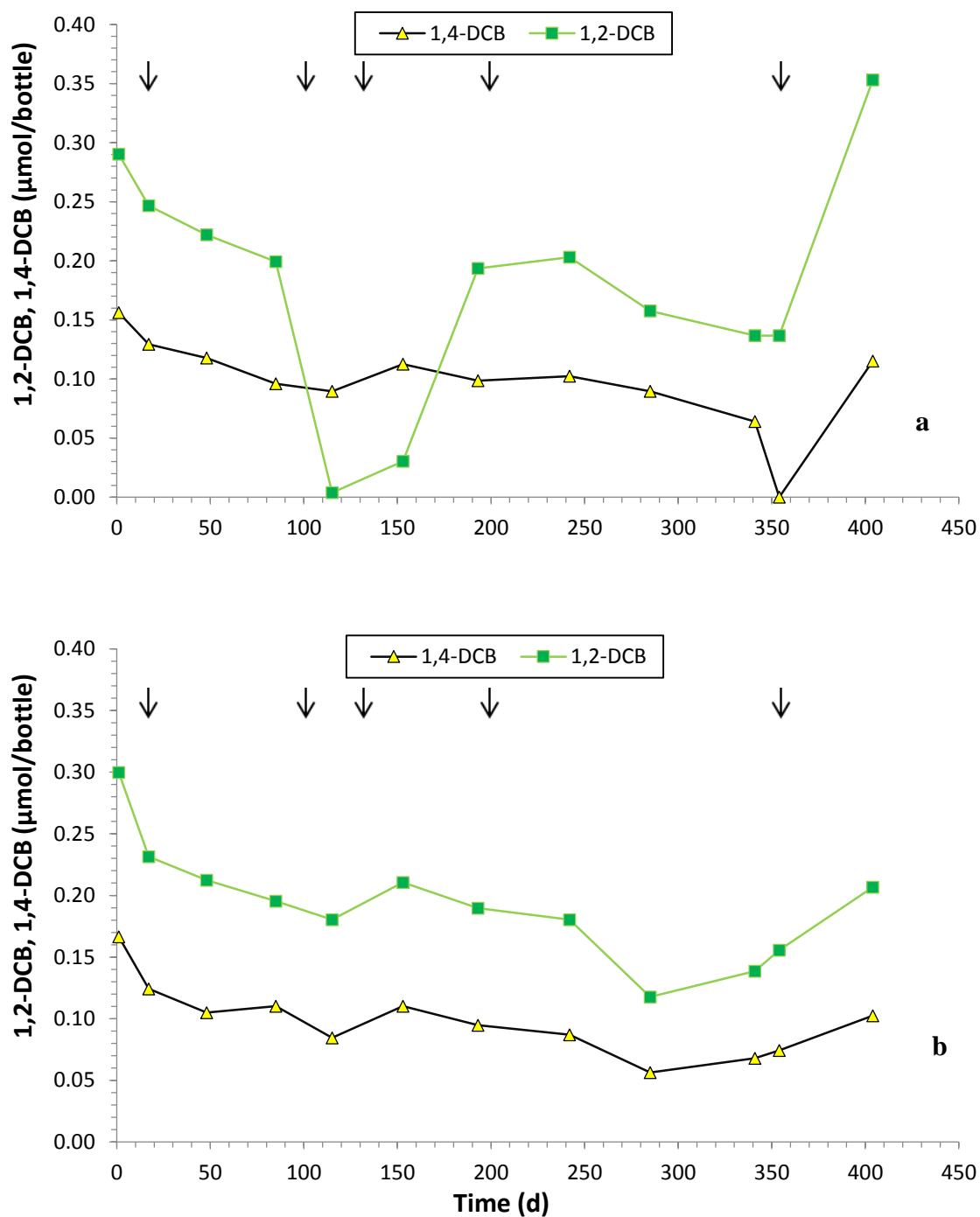


Figure C.13. Results for the ORP anaerobic lactate amended a) microcosm #1, low concentration chlorinated benzenes. b) Microcosm #2, low concentration chlorinated benzenes. Arrows indicate addition of lactate.

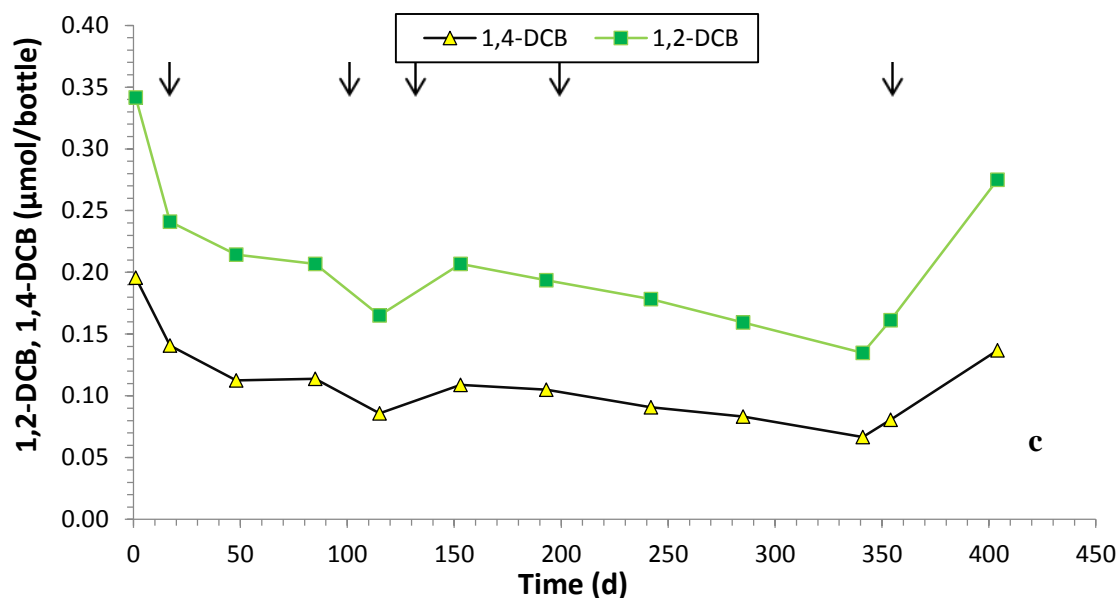


Figure C.13 (continued). Results for the ORP anaerobic lactate amended c) microcosm #3, low concentration chlorinated benzenes. Arrows indicate addition of lactate.

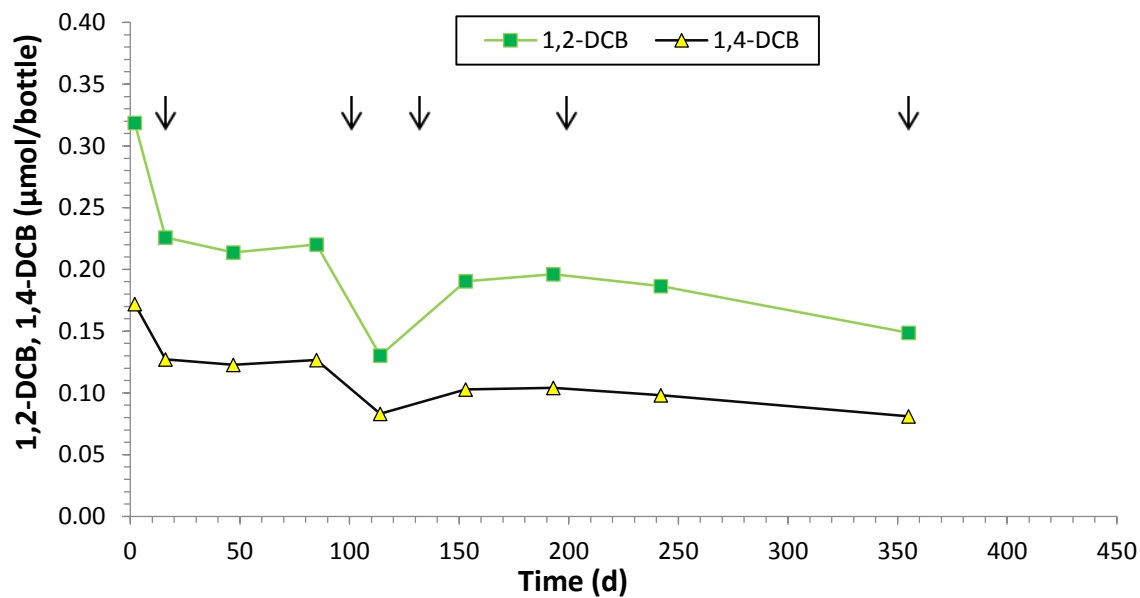


Figure C.14. Results for the triplicate ORP anaerobic EVO amended, low concentration chlorinated benzenes. Arrows indicate addition of EVO.

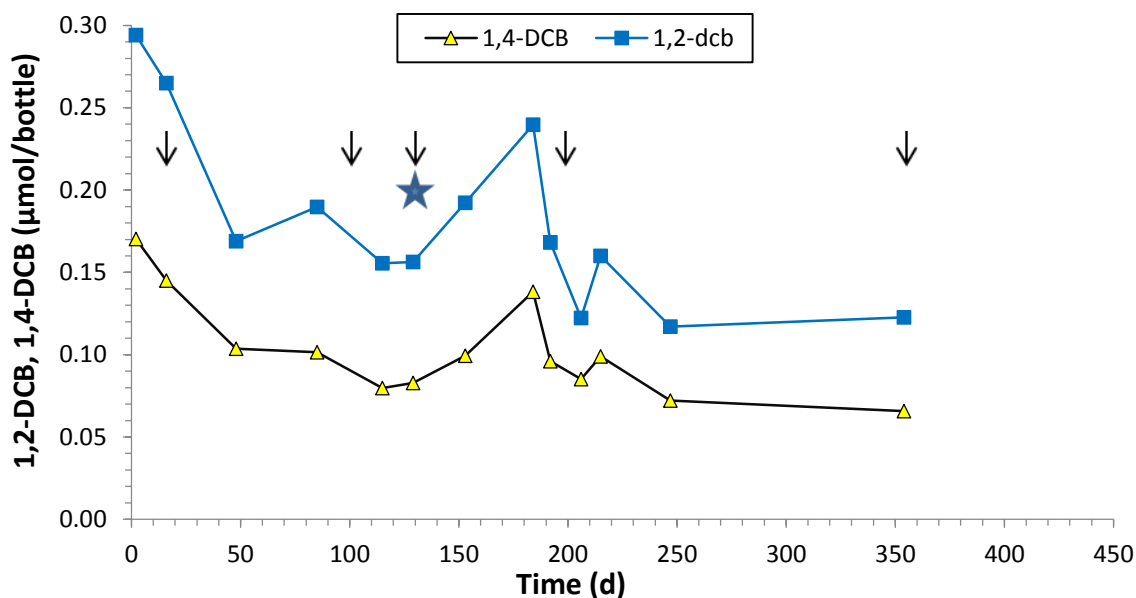


Figure C.15. Results for the triplicate ORP anaerobic bioaugmented, low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of KB-1 culture.

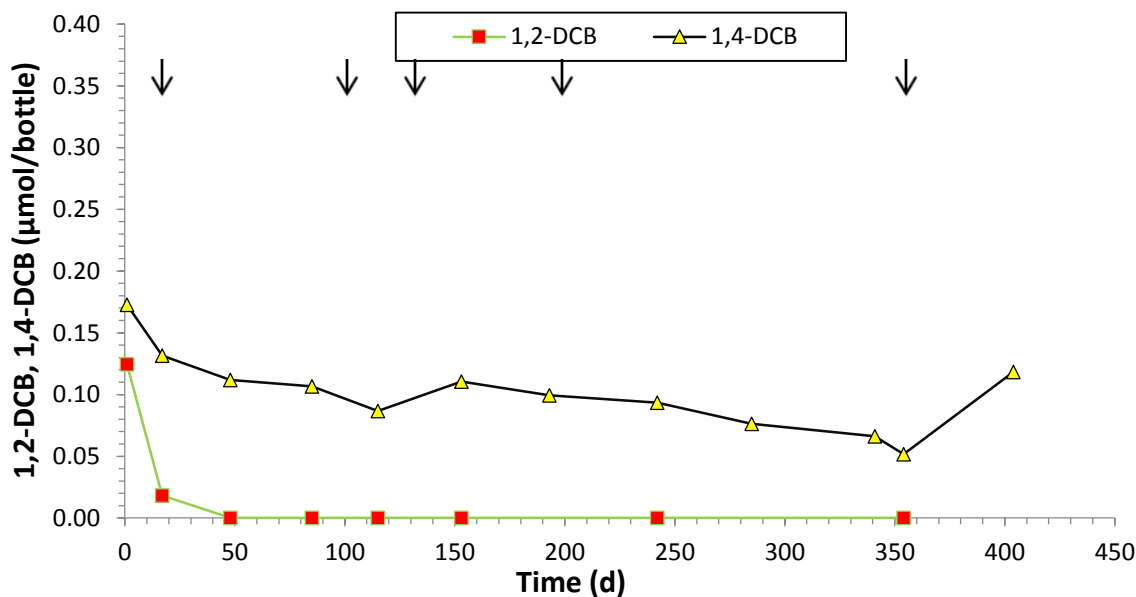


Figure C.16. Results for the triplicate ORP anaerobic ZVI and lactate amended microcosms, low concentration chlorinated benzenes. Arrows indicate addition of lactate.

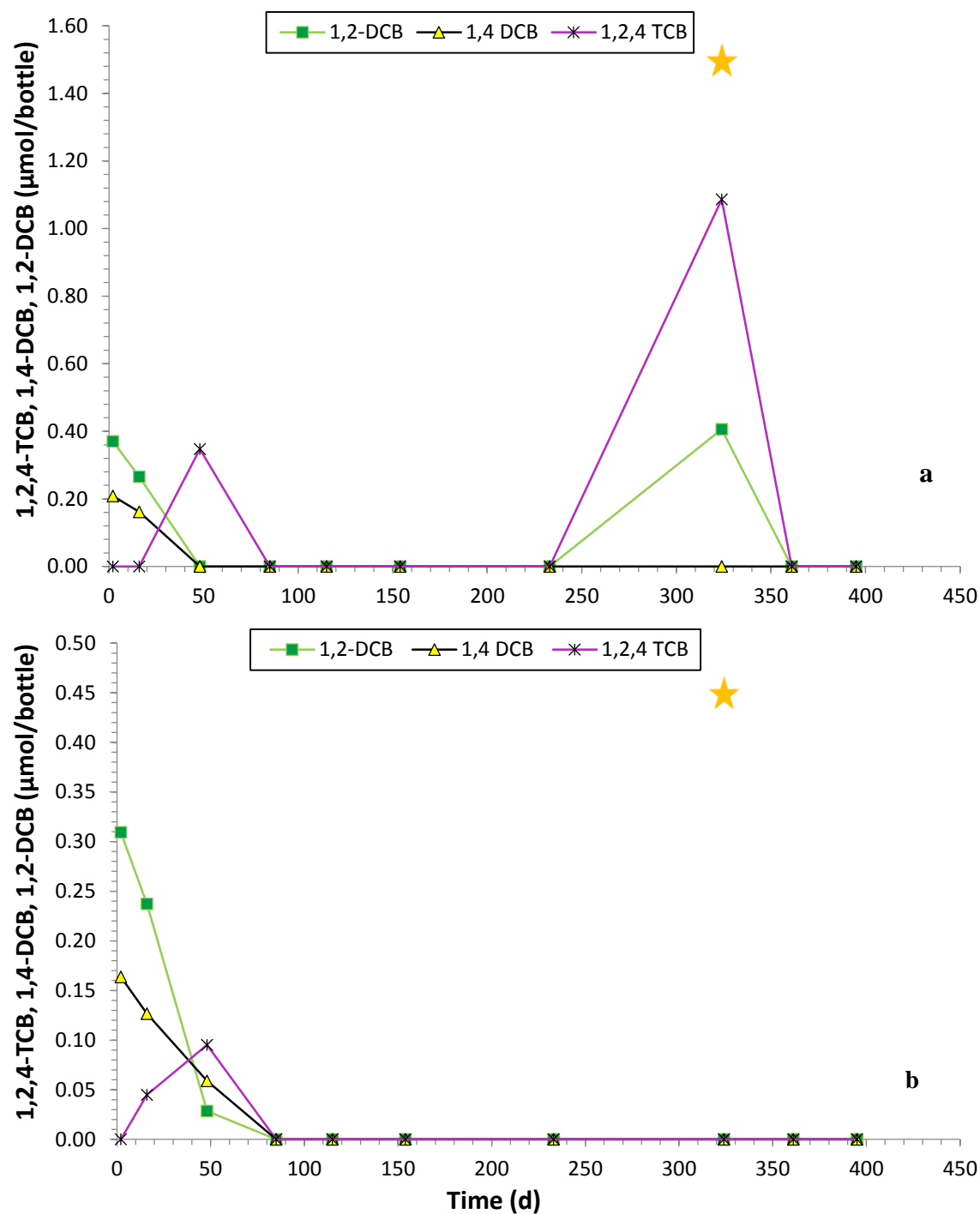


Figure C.17. Results for the ORP aerobic unamended a) microcosm #1, b) microcosm #2 low concentration chlorinated benzenes. Stars indicate addition of soil from WWTP-AER.

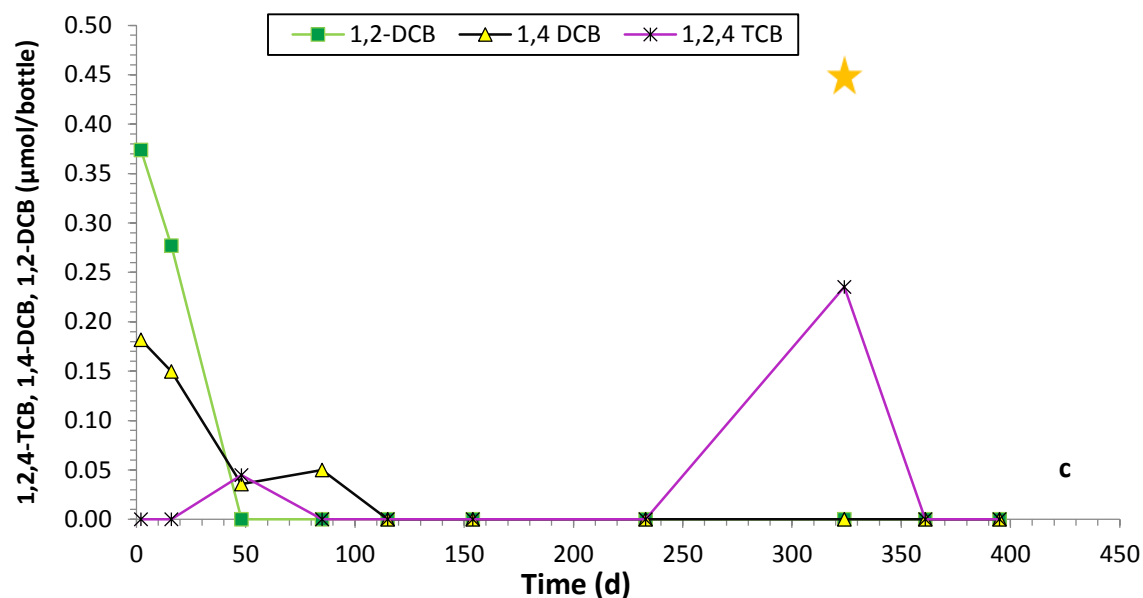


Figure C.17 (continued). Results for the ORP aerobic unamended c) microcosm #3, low concentration chlorinated benzenes. Stars indicate addition of soil from WWTP-AER.

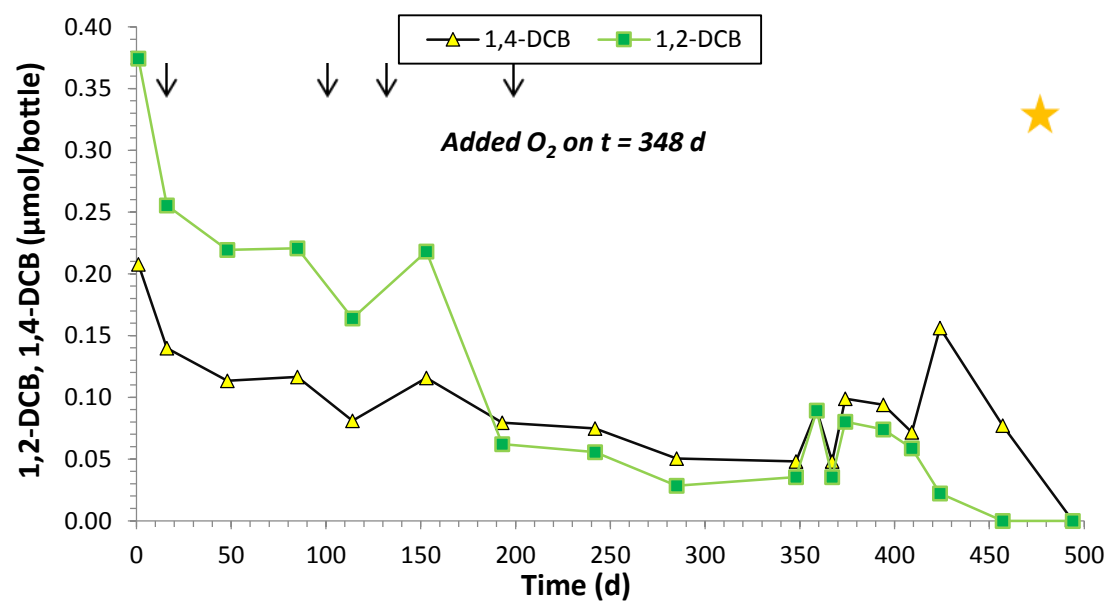


Figure C.18. Results for the triplicate ORP anaerobic/aerobic lactate amended microcosms, low concentration chlorinated benzenes. Arrows indicate addition of lactate.

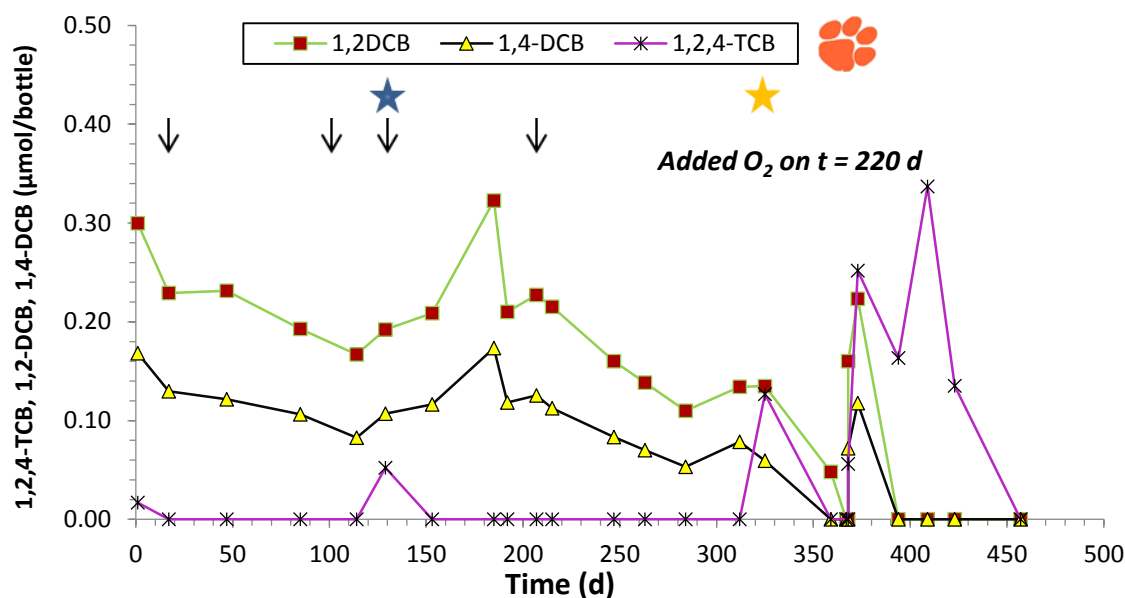


Figure C.19. Results for the triplicate ORP anaerobic/aerobic bioaugmented amended microcosms, low concentration chlorinated benzenes. Arrows indicate addition of lactate. Blue stars indicate addition of KB-1 culture. Yellow stars indicate addition of soil from WWTP-AER. Tiger paws indicate re-addition of chlorinated benzenes.

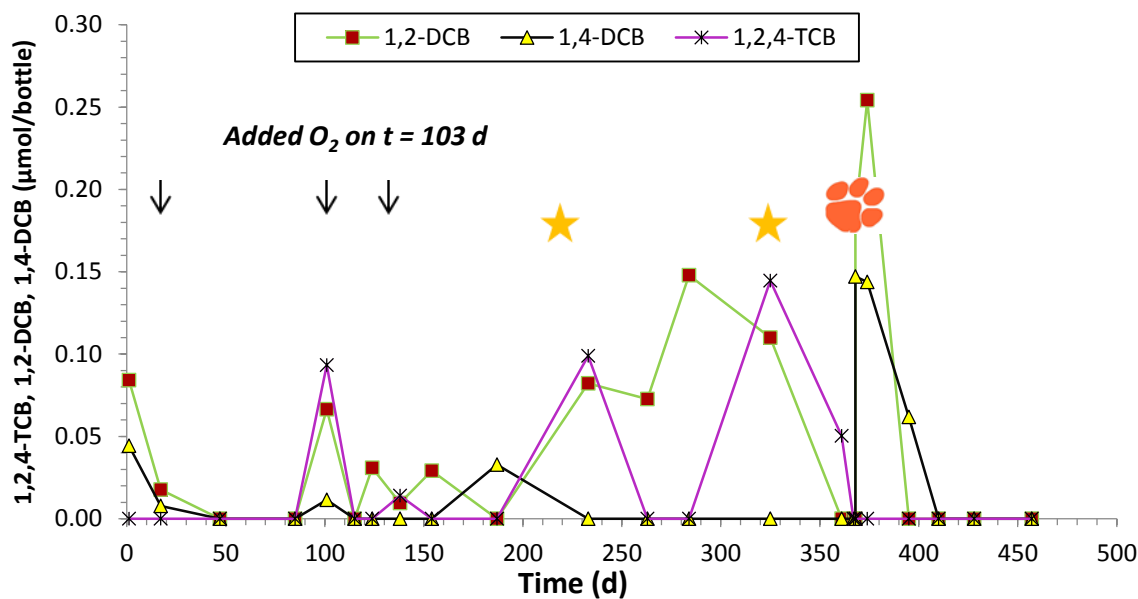


Figure C.20. Results for the triplicate ORP anaerobic/aerobic ZVI and lactate amended microcosms, low concentration chlorinated benzenes. Arrows indicate addition of lactate. Yellow stars indicate addition of soil from WWTP-AER. Tiger paws indicate re-addition of chlorinated benzenes.

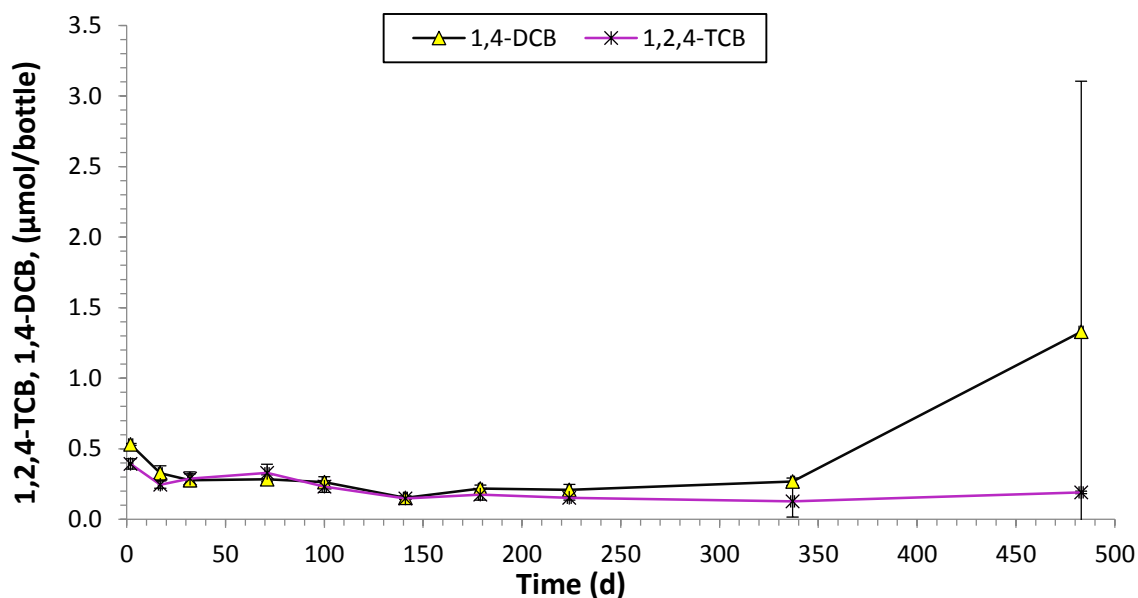


Figure C.21. Results for the triplicate tank farm autoclaved control microcosms; low concentration chlorinated benzenes. Error bars represent standard deviations.

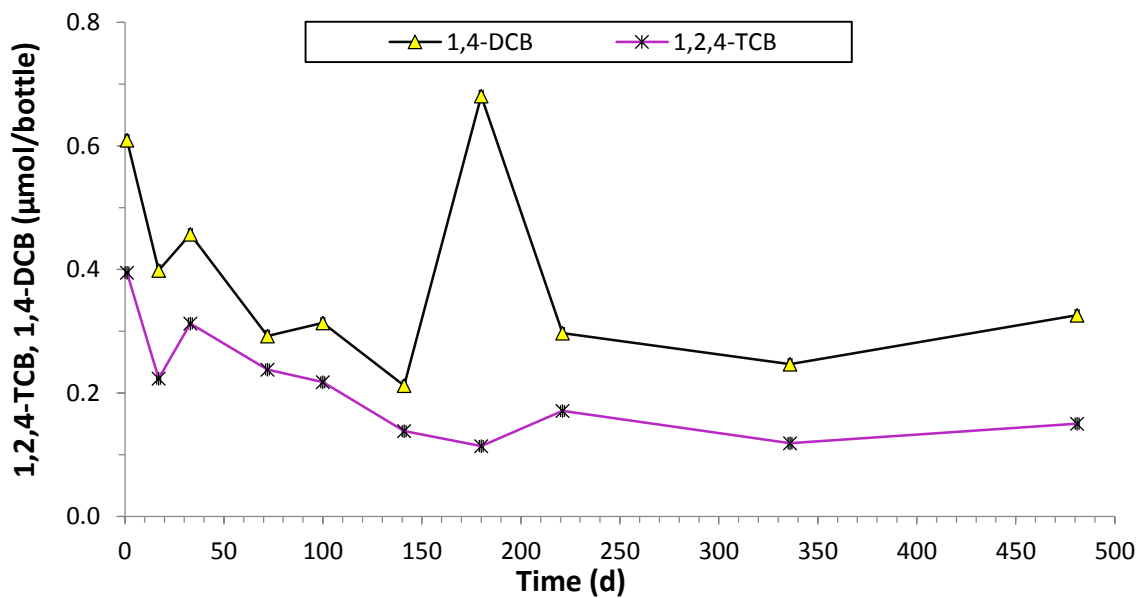


Figure C.22. Results for the triplicate tank farm anaerobic unamended microcosms; low concentration chlorinated benzenes.

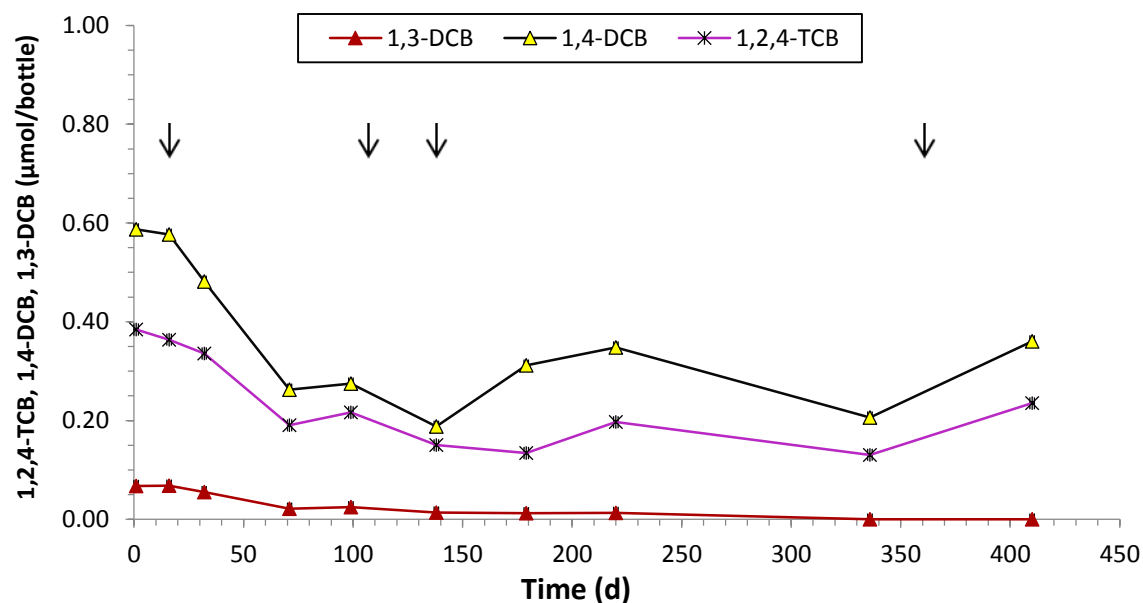


Figure C.23. Results for the triplicate tank farm anaerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

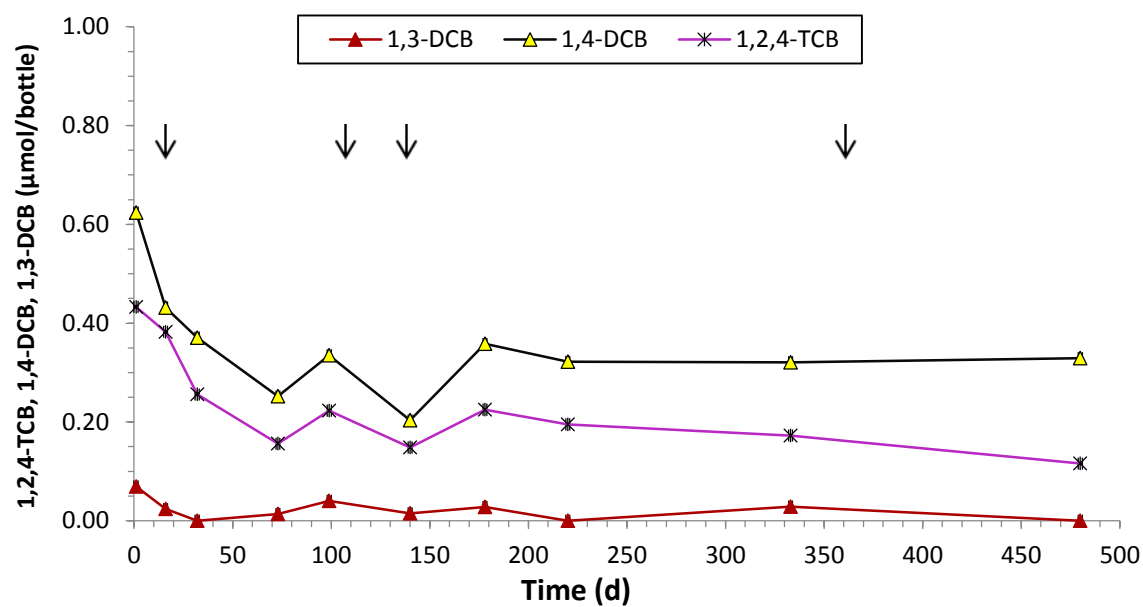


Figure C.24. Results for the triplicate tank farm anaerobic EVO amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of EVO.

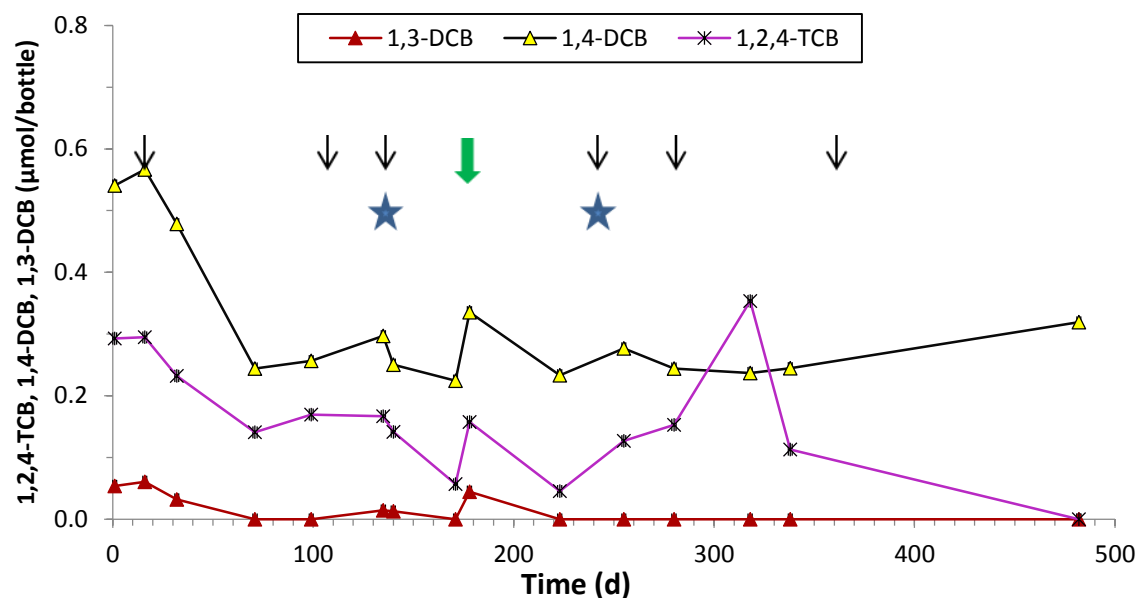


Figure C.25. Results for the triplicate tank farm anaerobic bioaugmented microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate. Green arrows indicate addition of corn syrup. Stars indicate addition of KB-1 culture.

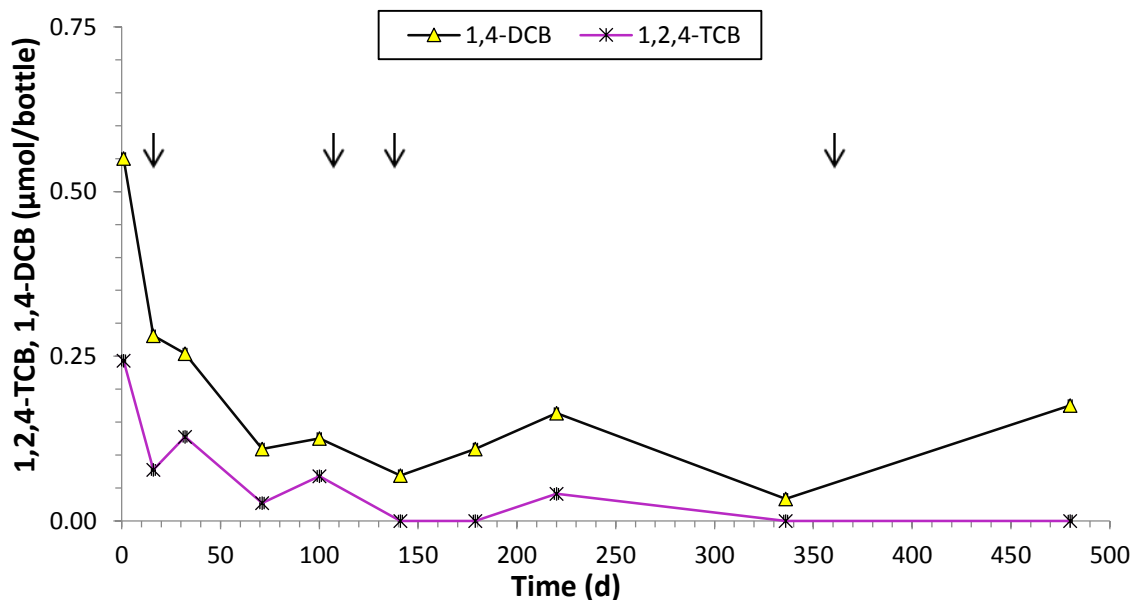


Figure C.26. Results for the triplicate tank farm anaerobic ZVI and lactate amended microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate.

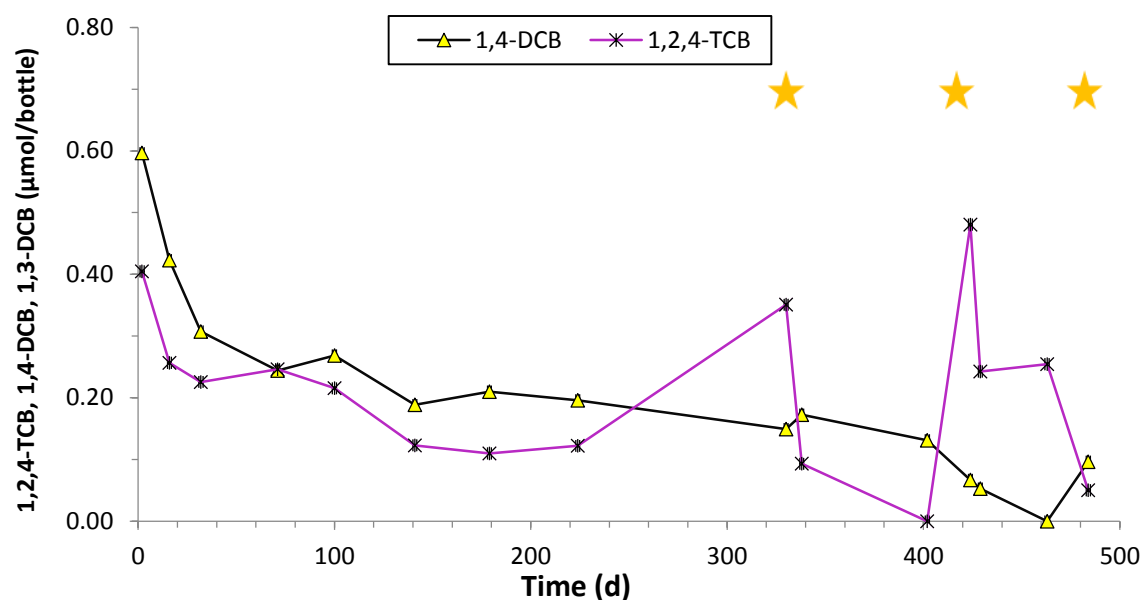


Figure C.27. Results for the triplicate tank farm aerobic unamended microcosms; low concentration chlorinated benzenes. Stars indicate addition of soil from WWTP-AER.

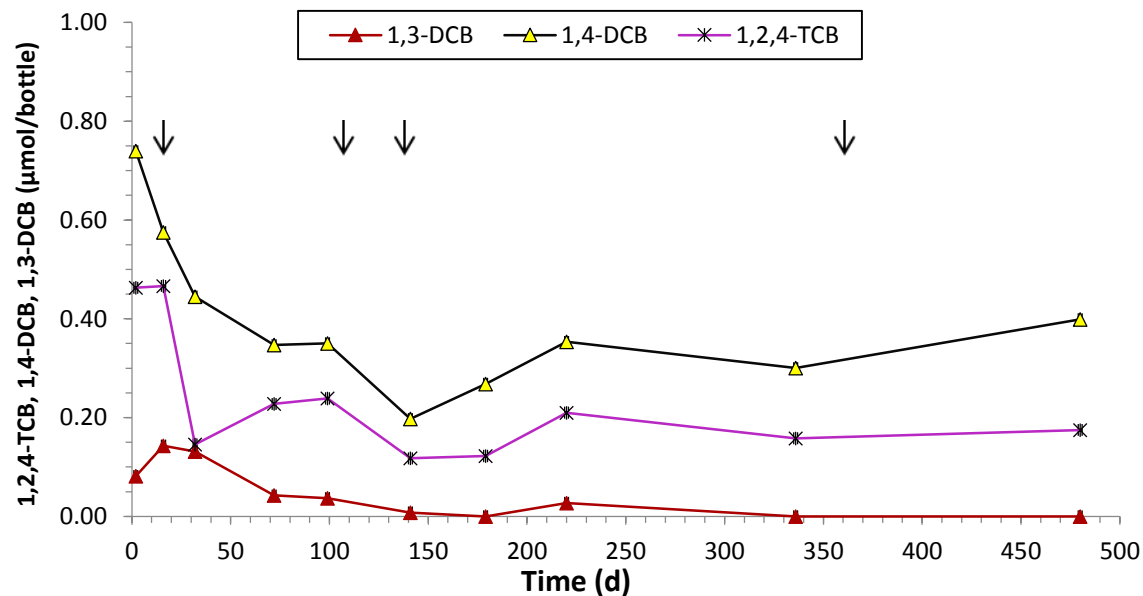


Figure C.28. Results for the triplicate tank farm anaerobic/aerobic lactate amended microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate.

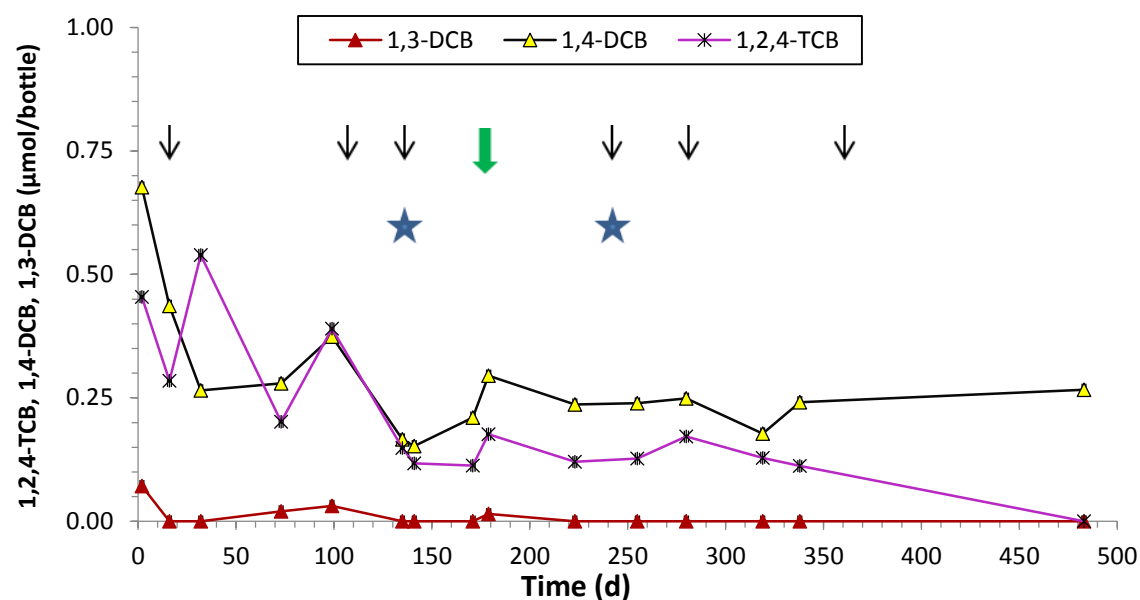


Figure C.29. Results for the triplicate tank farm anaerobic/aerobic bioaugmented microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate. Green arrows indicate addition of corn syrup. Stars indicate addition of KB-1 culture.

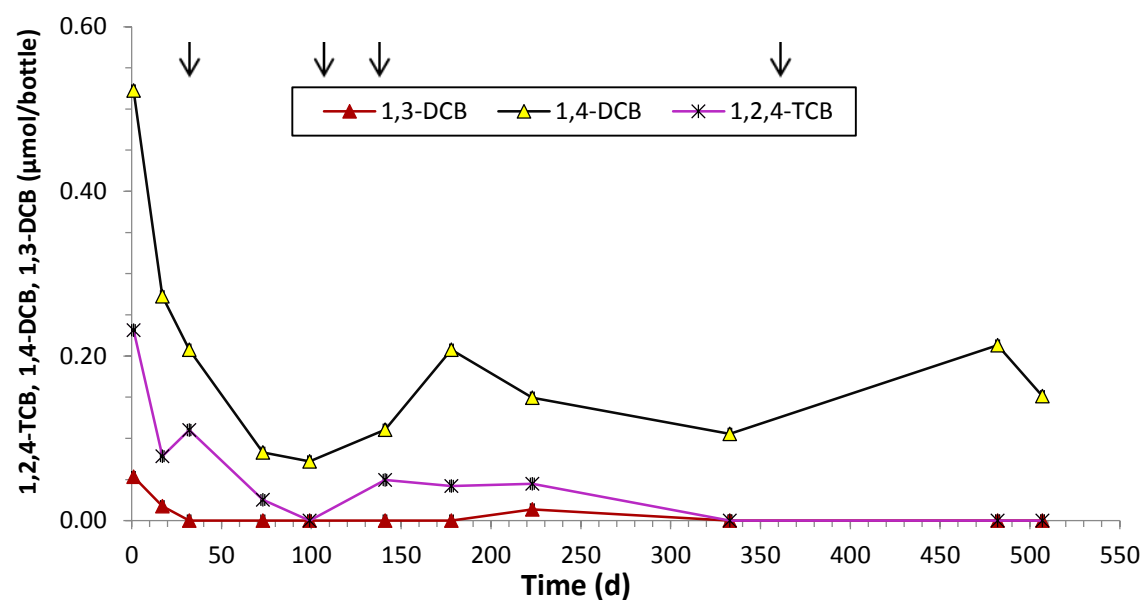


Figure C.30. Results for the triplicate tank farm anaerobic ZVI and lactate amended microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate.

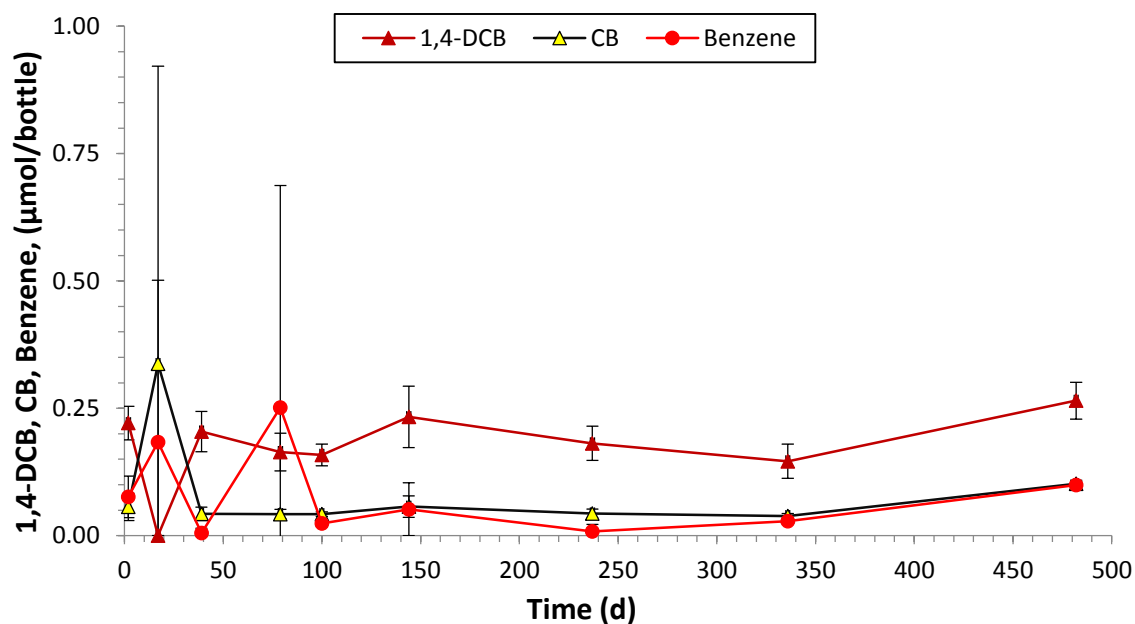


Figure C.31. Results for the triplicate grease trap autoclaved control microcosms; low concentration chlorinated benzenes. Error bars represent standard deviations.

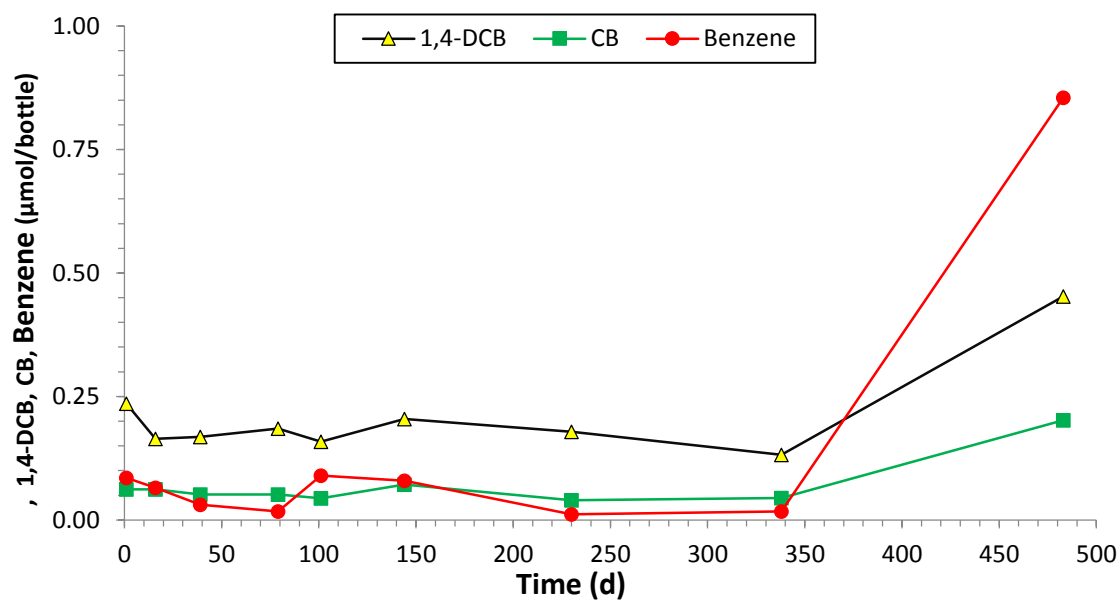


Figure C.32. Results for the triplicate grease trap anaerobic unamended microcosms; low concentration chlorinated benzenes.

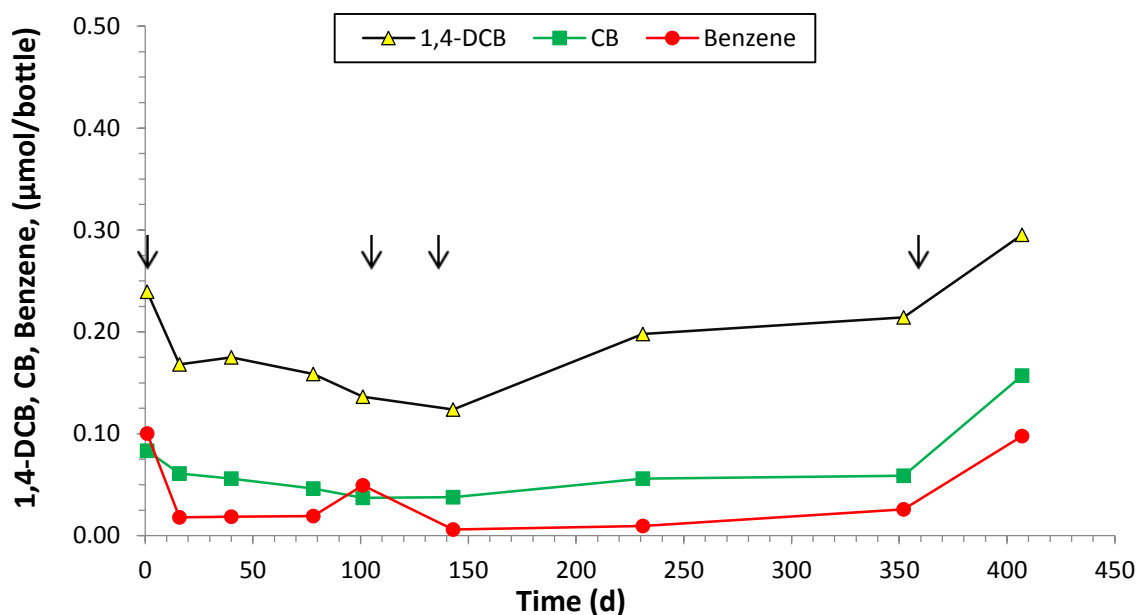


Figure C.33. Results for the triplicate grease trap anaerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

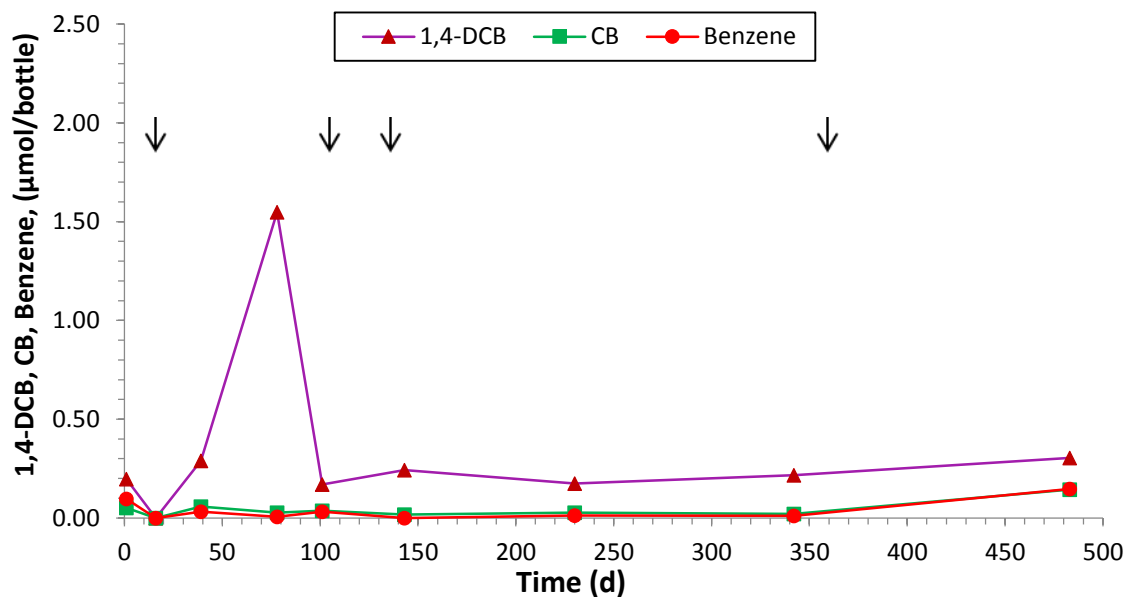


Figure C.34. Results for the triplicate grease trap anaerobic EVO amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of EVO.

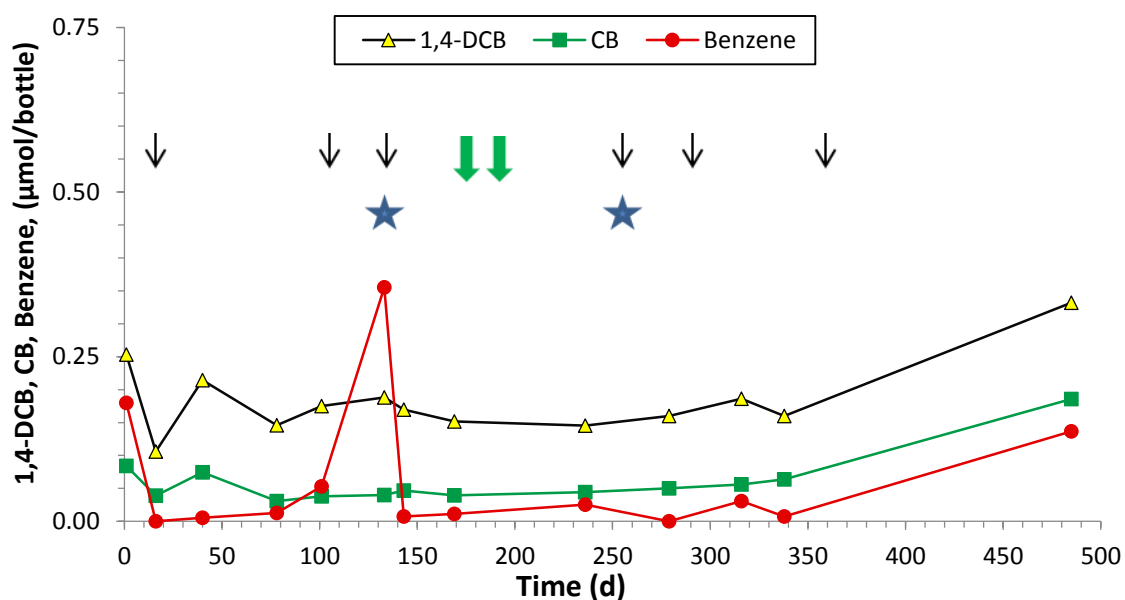


Figure C.35. Results for the triplicate grease trap anaerobic bioaugmented microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate. Green arrows indicate corn syrup. Stars indicate addition of KB-1 culture.

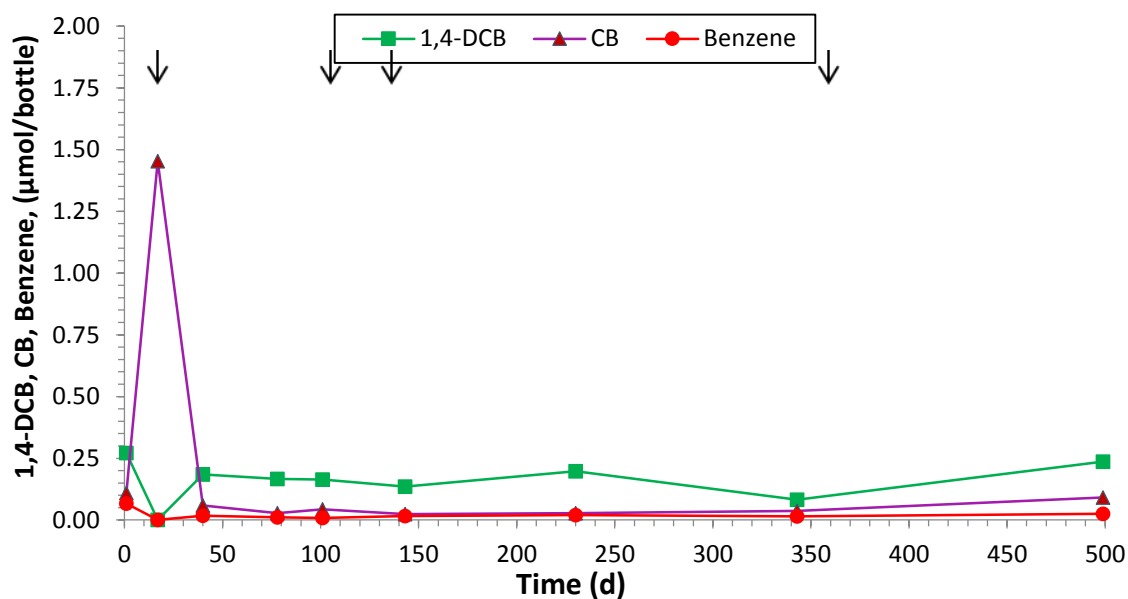


Figure C.36. Results for the triplicate grease trap anaerobic ZVI and lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

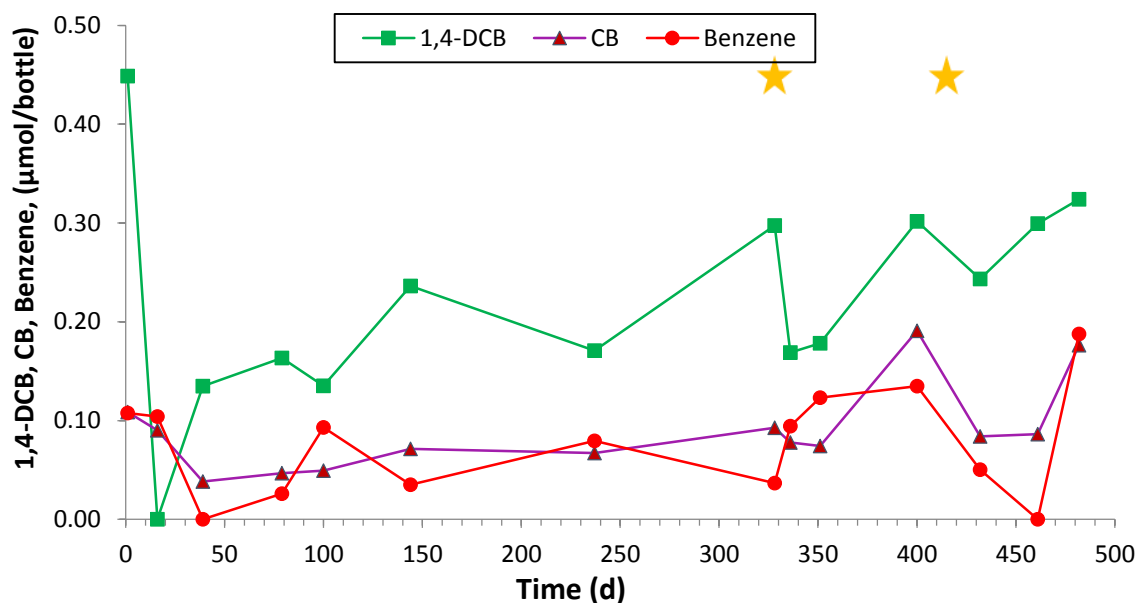


Figure C.37. Results for the triplicate grease trap aerobic unamended microcosms; low concentration chlorinated benzenes. Stars indicate addition of soil from WWTP-aer.

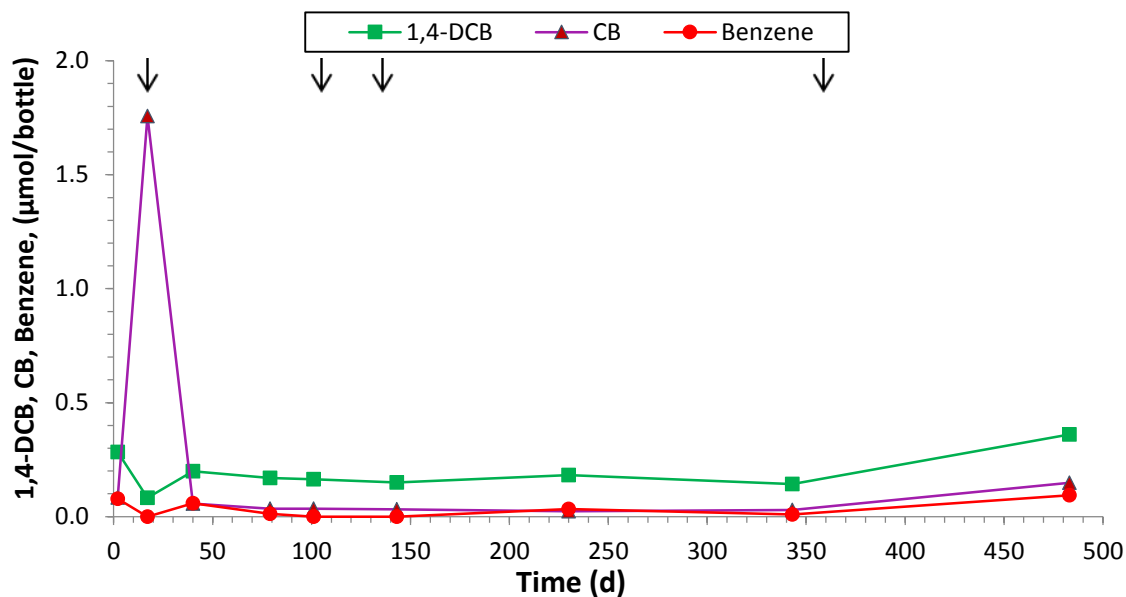


Figure C.38. Results for the triplicate grease trap anaerobic/aerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

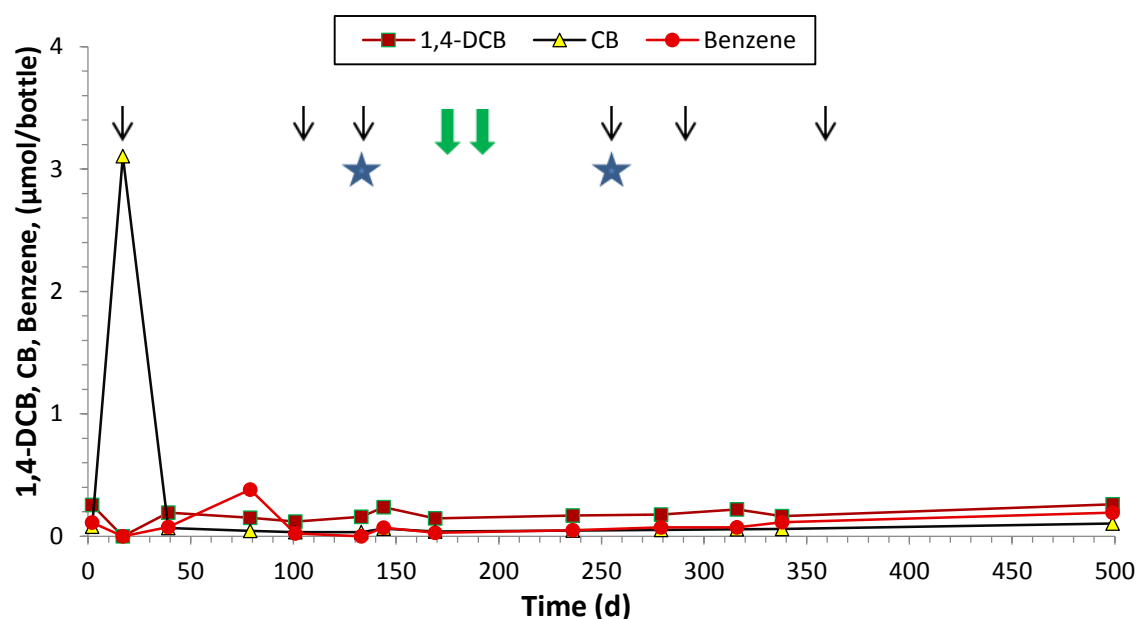


Figure C.39. Results for the triplicate grease trap anaerobic/aerobic bioaugmented microcosms; low concentration chlorinated benzenes. Black arrows indicate addition of lactate. Green arrows indicate corn syrup. Stars indicate addition of KB-1 culture.

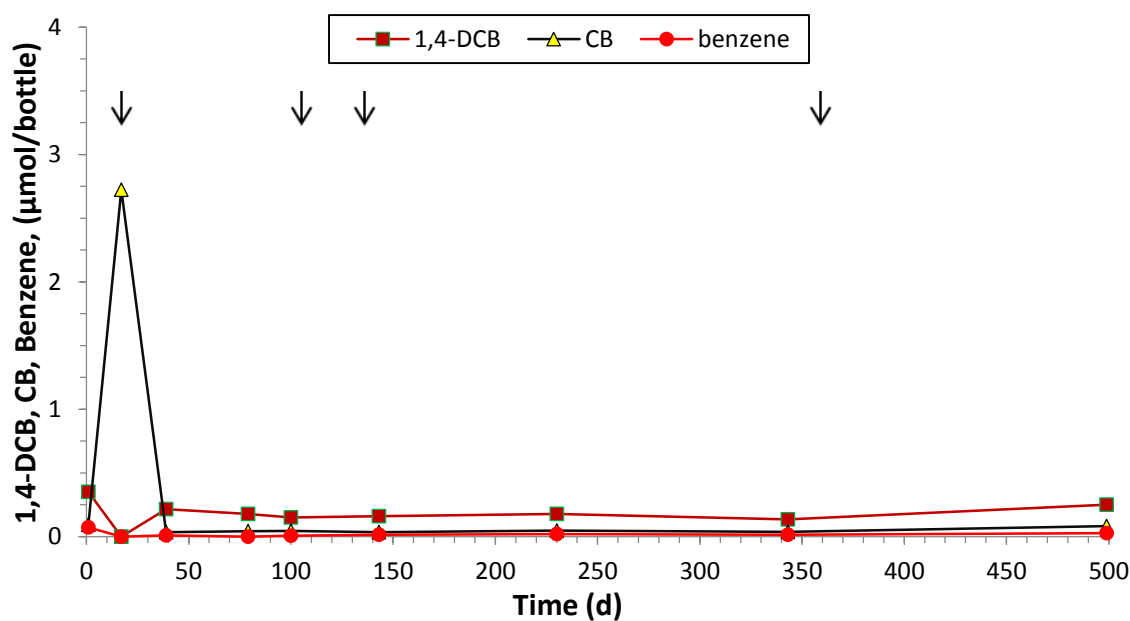


Figure C.40. Results for the triplicate grease trap anaerobic/aerobic ZVI and lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

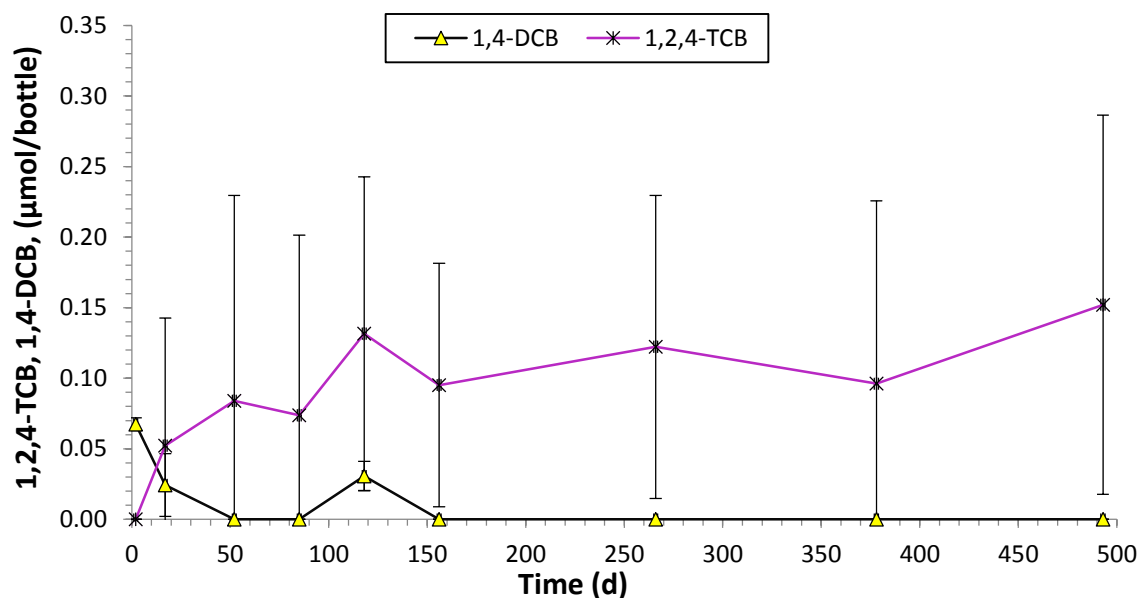


Figure C.41. Results for the triplicate downgradient plume autoclaved control microcosms; low concentration chlorinated benzenes. Error bars represent standard deviations.

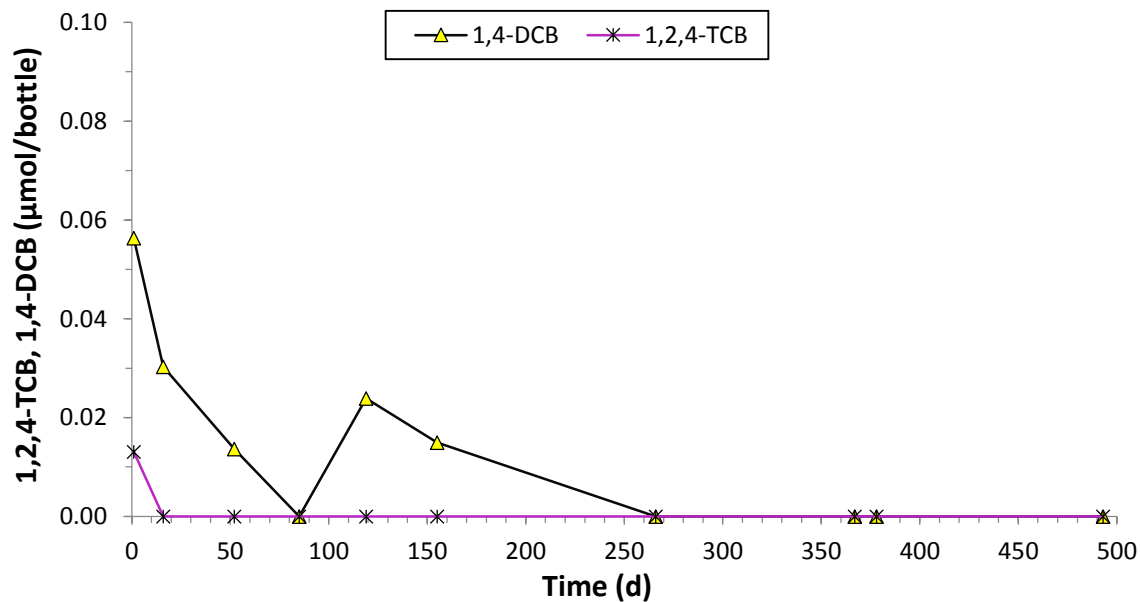


Figure C.42. Results for the triplicate downgradient plume anaerobic unamended microcosms; low concentration chlorinated benzenes.

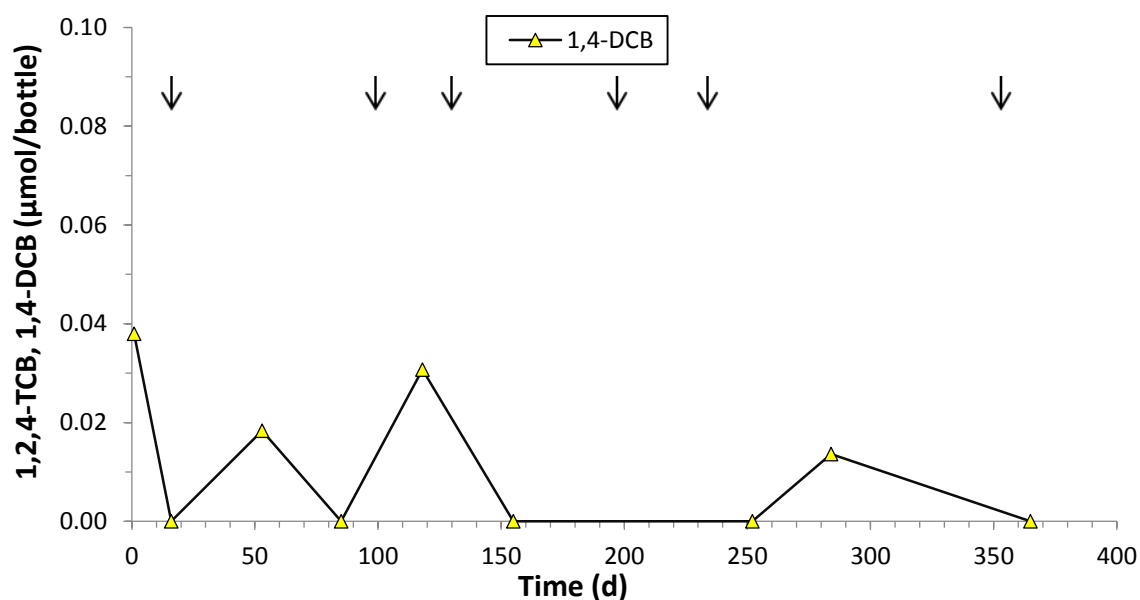


Figure C.43. Results for the triplicate downgradient plume anaerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

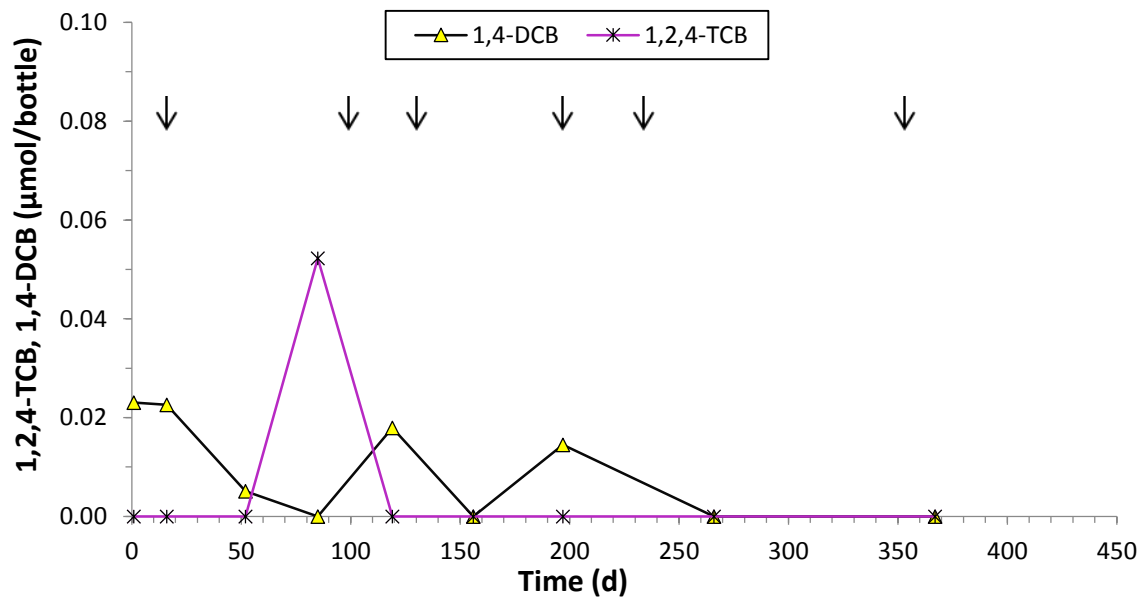


Figure C.44. Results for the triplicate downgradient plume anaerobic EVO amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of EVO.

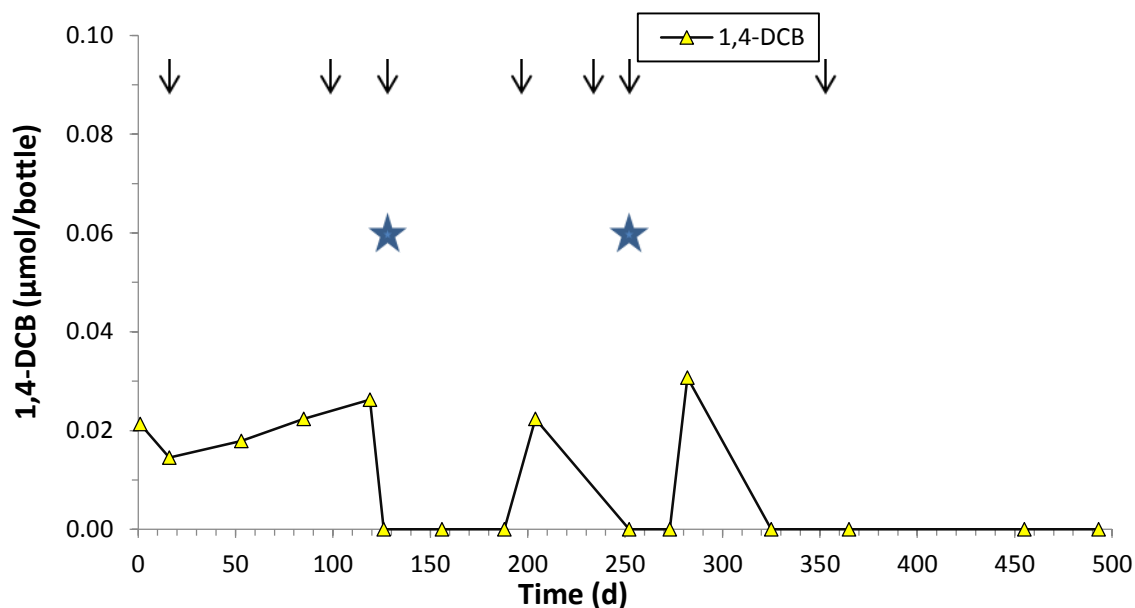


Figure C.45. Results for the triplicate downgradient plume anaerobic bioaugmented microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Stars indicate addition of KB-1 culture.

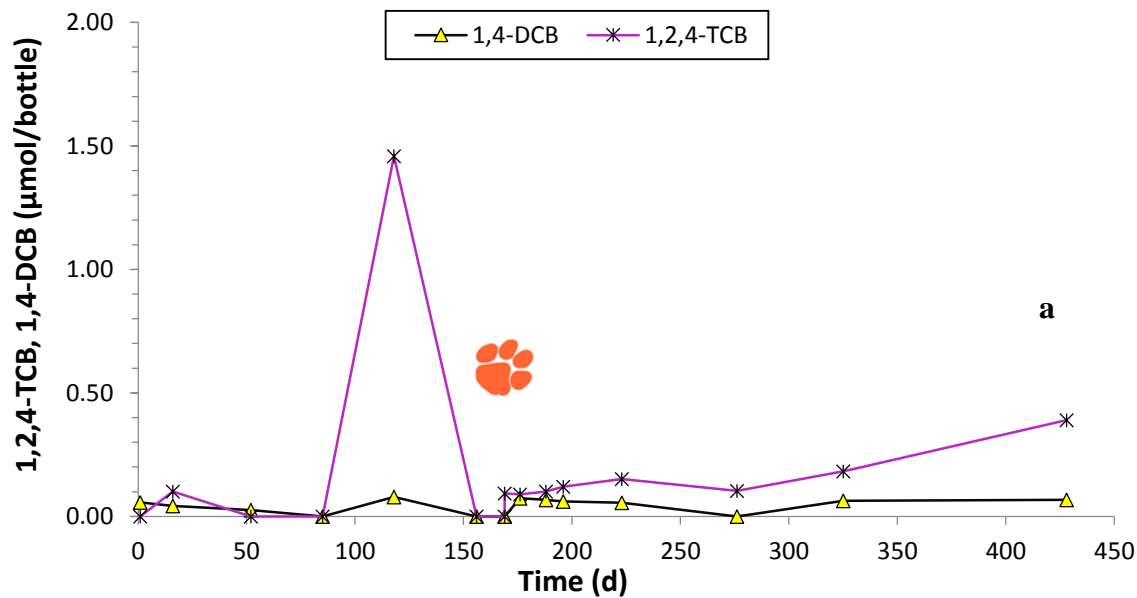


Figure C.46. Results for the downgradient plume aerobic unamended a) microcosm #1; low concentration chlorinated benzenes. Tiger paw indicate re-addition of chlorinated benzenes.

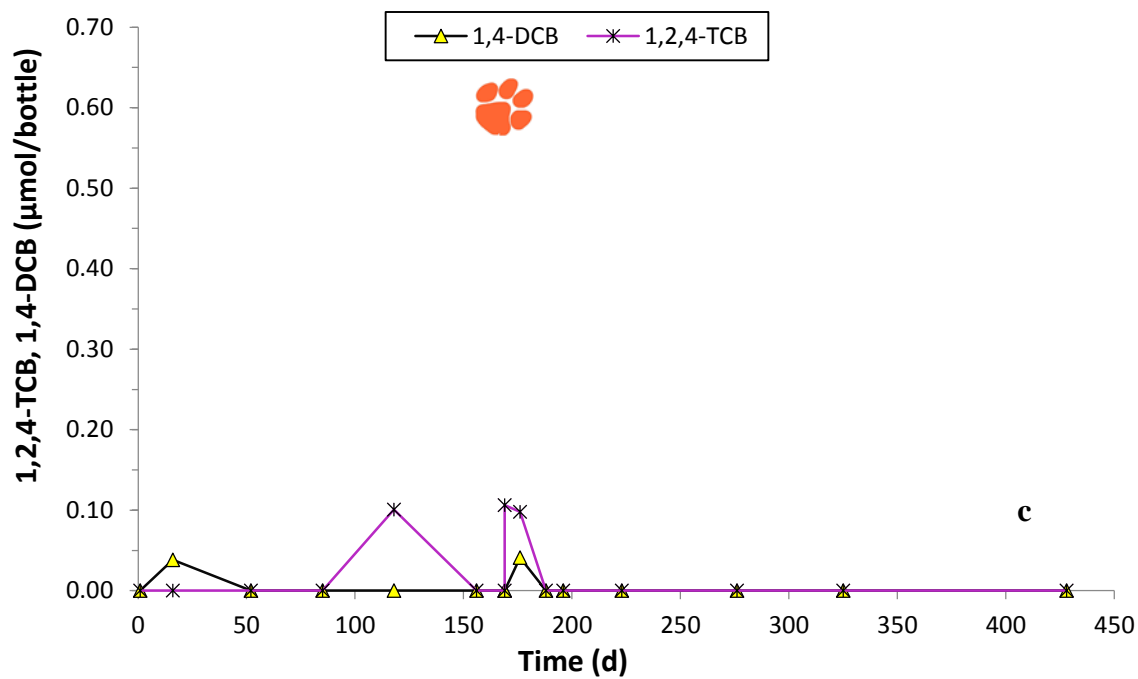
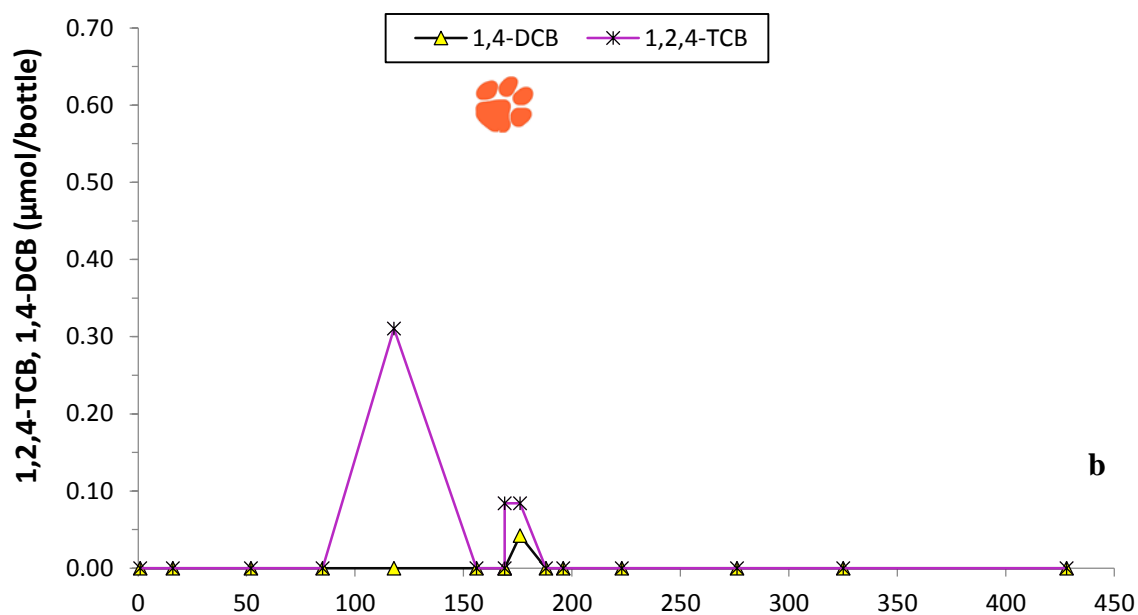


Figure C.46 (continued). Results for the downgradient plume aerobic unamended b) microcosm #2 and c) microcosm #3; low concentration chlorinated benzenes. Tiger paw indicate re-addition of chlorinated benzenes.

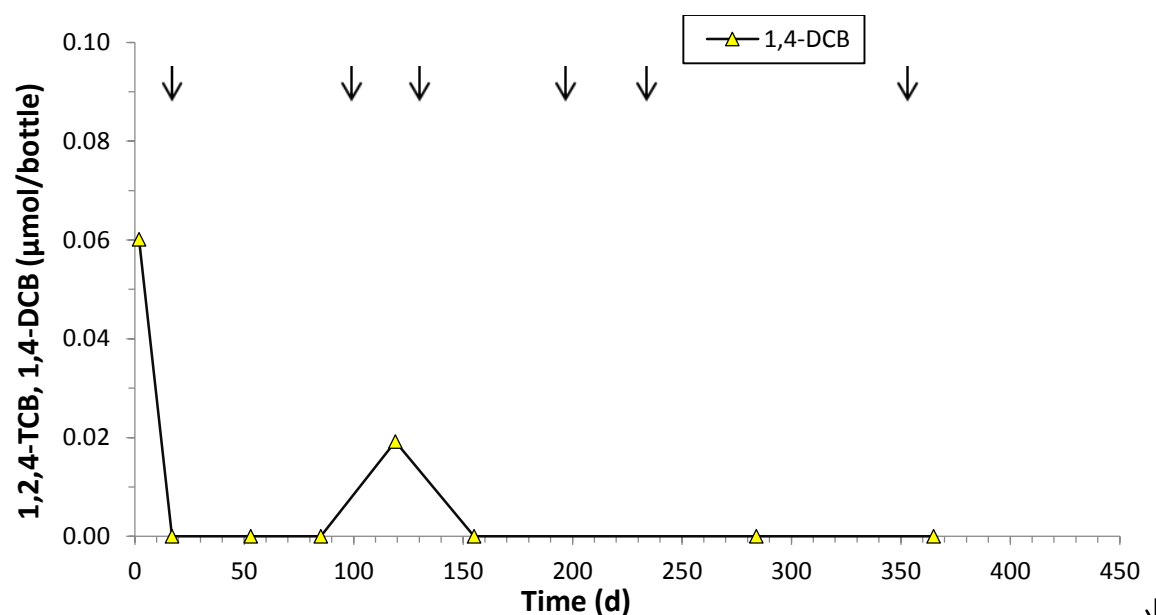


Figure C.47. Results for the triplicate downgradient plume anaerobic/aerobic lactate amended microcosms; low concentration chlorinated benzenes. Arrows indicate addition of lactate.

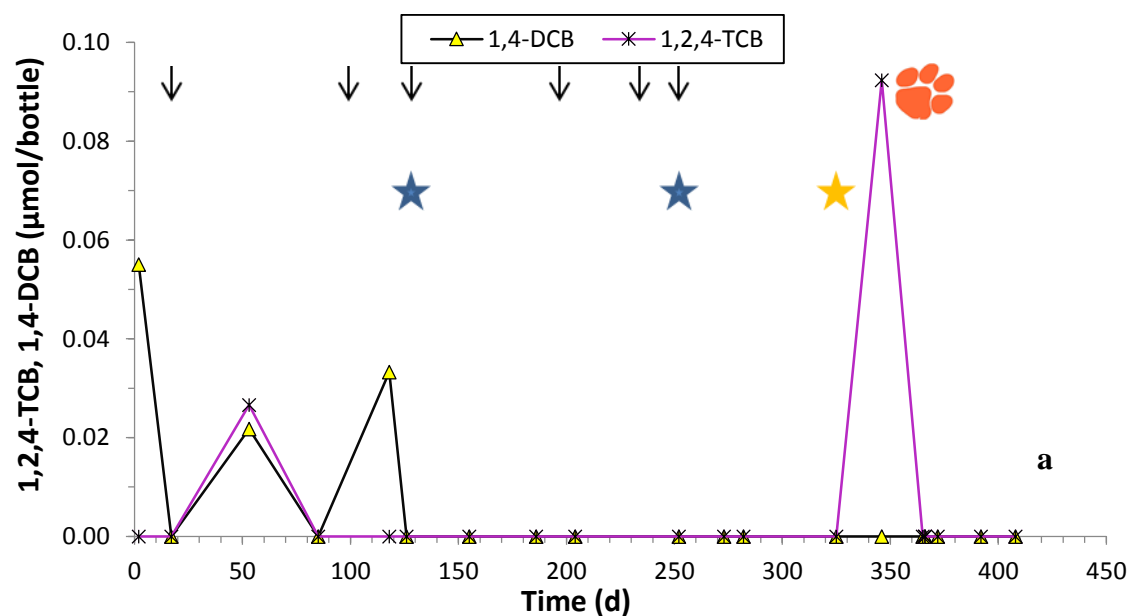


Figure C.48. Results for the downgradient plume anaerobic/aerobic bioaugmented a) microcosm #1; low concentration chlorinated benzenes. Arrows indicate addition of lactate. Yellow stars indicate addition of soil from WWTP-aer. Blue stars indicate addition of KB-1 culture. Tiger paw indicate re-addition of chlorinated benzenes.

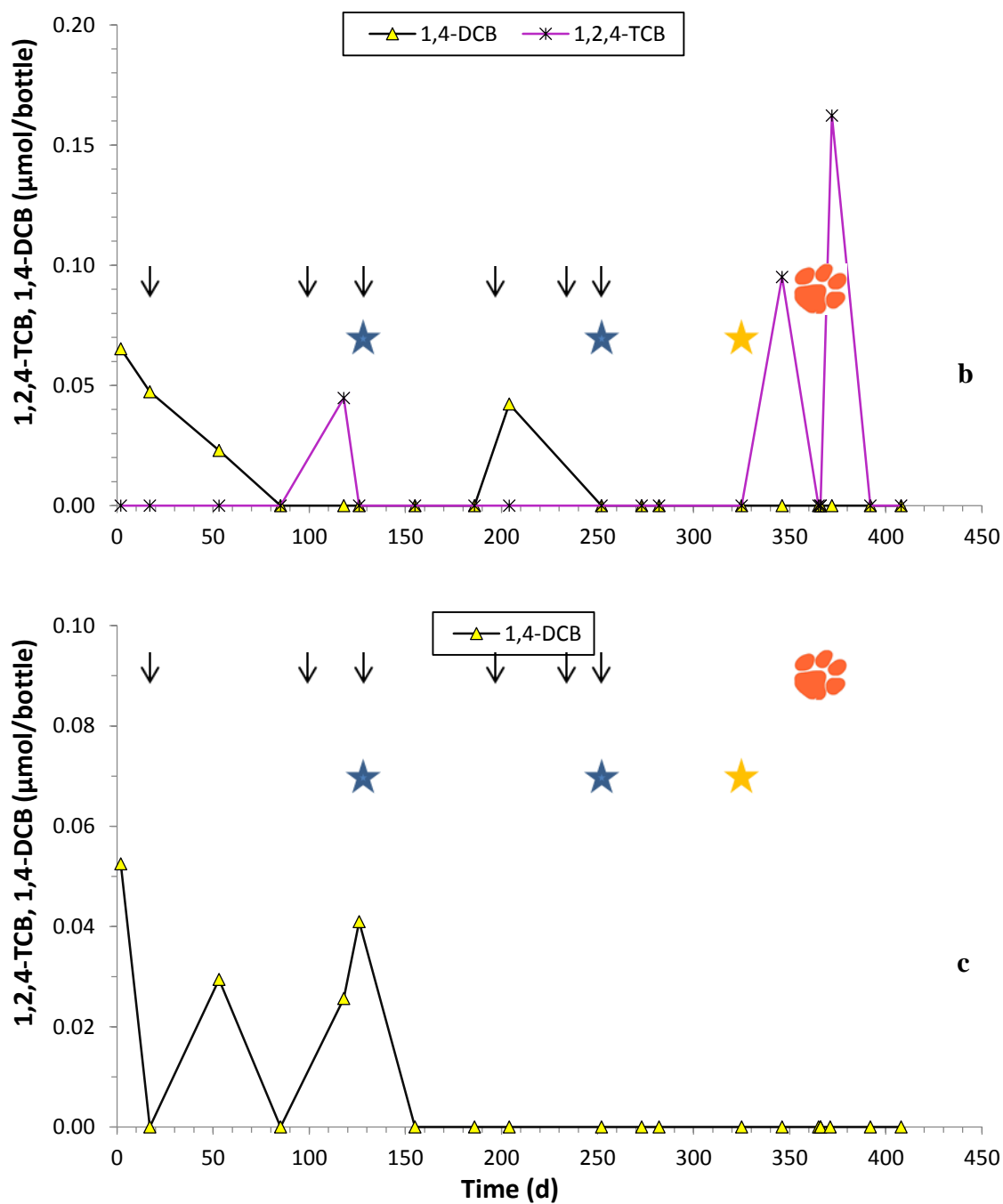


Figure C.48 (continued). Results for the downgradient plume anaerobic/aerobic bioaugmented microcosm #2 b) and microcosm #3 c); low concentration chlorinated benzenes. Arrows indicate addition of lactate. Yellow stars indicate addition of soil from WWTP-aer. Blue stars indicate addition of KB-1 culture. Tiger paw indicate re-addition of chlorinated benzenes.

REFERENCES

- Anderson RT, Lovley DR (2000) Anaerobic bioremediation of benzene under sulfate-reducing conditions in a petroleum-contaminated aquifer. *Environ Sci Technol* 34(11):2261-2266
- Anderson RT, Rooney-Varga JN, Gaw CV, Lovley DR (1998) Anaerobic benzene oxidation in the Fe(III) reduction zone of petroleum-contaminated aquifers. *Environ Sci Technol* 32(9):1222-1229
- Beeman RE, Bleckmann CA (2002) Sequential anaerobic–aerobic treatment of an aquifer contaminated by halogenated organics: field results. *J Contam Hydrol* 57:147-159
- Beurskens JEM, Dekker CGC, van den Heuvel H, Swart M, De Wolf J (1994) Dechlorination of chlorinated benzenes by an anaerobic microbial consortium that selectively mediates the thermodynamic most favorable reactions. *Environ Sci Technol* 28(4):701-706
- Bosma TNP, van der Meer JR, Schraa G, Tros ME, Zehnder JB (1988) Reductive dechlorination of all trichloro- and dichlorobenzene isomers. *FEMS Microbiol Ecol* 53:223-229
- Burns M, Sublette KL, Sobieraj J, Ogles D, Koenigsberg S (2013) Concurrent and complete anaerobic reduction and microaerophilic degradation of mono-, di-, and trichlorobenzenes. *Remediation J* 23(3):37-53
- Cashwell JM, Marotte R, Willis S, Clarke J, McLaughlin D, Freedman DL (2004) Evaluation of cultures for bioaugmentation of a PCE plume. In: *Proceedings of the Battelle Fourth International Conference on remediation of chlorinated and recalcitrant compounds*, Monterey, CA,. Battelle Press, Columbus, OH.
- Chakraborty R, Coates JD (2005) Hydroxylation and carboxylation--two crucial steps of anaerobic benzene degradation by *Dechloromonas* strain RCB. *Appl Environ Microbiol* 71(9):5427-5432
- Coleman NV, Mattes TE, Gossett JM, Spain JC (2002) Phylogenetic and kinetic diversity of aerobic vinyl chloride-assimilating bacteria from contaminated sites. *Appl Environ Microbiol* 68(12):6162-6171
- De Bruin WP, Kotterman MJJ, Maarten A, Schraa G, Zehnder AJB (1992) Complete biological reductive transformation of tetrachloroethene to ethane. *Appl Environ Microb* 58(6):1996-2000
- Devlin JF, Katic D, Barker JF (2004) In situ sequenced bioremediation of mixed contaminants in groundwater. *J Contam Hydrol* 69 (3-4):233-261

- Elango V (2010) Biodegradation and bioremediation of hexachlorocyclohexane isomers, chlorinated ethenes, chlorinated benzenes and benzene. Ph.D. Thesis, Clemson University, Clemson, SC
- Elango V, Cashwell JM, Bellotti MJ, Marotte R, Freedman DL (2010) Bioremediation of hexachlorocyclohexane isomers, chlorinated benzenes, and chlorinated ethenes in soil and fractured dolomite. *Bioremediation J* 14(1):10 - 27
- Elango V, Kurtz HD, Freedman DL (2011) Aerobic cometabolism of trichloroethene and *cis*-dichloroethene with benzene and chlorinated benzenes as growth substrates. *Chemosphere* 84(2):247-253
- Ellis DE, Lutz EJ, Odom JM, Buchanan RJJ, Bartlett CL, Lee MD, Harkness MR, Deweerd KA (2000) Bioaugmentation for accelerated in situ anaerobic bioremediation. *Environ Sci Technol* 34(11):2254-2260
- Fu FL, Dionysiou DD, Liu H (2014) The use of zero-valent iron for groundwater remediation and wastewater treatment: a review. *J Hazard Mater* 267(28) 194-205
- Freedman DL, Gossett JM (1989) Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. *Appl Environ Microbiol* 55(9):2144-2151
- Fung JM, Weisenstein BP, Mack EE, Vidumsky JE, Ei TA, Zinder SH (2009) Reductive dehalogenation of dichlorobenzenes and monochlorobenzene to benzene in microcosms. *Environ Sci Technol* 43(7):2302-2307
- Giddings CGS, Liu F, Gossett JM (2010) Microcosm assessment of *Polaromonas* sp. JS666 as a bioaugmentation agent for degradation of *cis*-1,2-dichloroethene in aerobic, subsurface environments. *Ground Water Monit R* 30(2):106-113
- Gossett JM (1987) Measurement of Henry's Law constants for C₁ and C₂ chlorinated hydrocarbons. *Environ Sci Technol* 21(12):202-208
- Gossett JM (2010) Sustained aerobic oxidation of vinyl chloride at low oxygen concentrations. *Environ Sci Technol* 44(4):1405-1411
- Hartmans S, de Bont JAM (1992) Aerobic vinyl chloride metabolism in *Mycobacterium aurum* L1. *Appl Environ Microbiol* 58(4):1220-1226
- Huling SG, Pivets BE (2006) In-Situ Chemical Oxidation. Environmental Protection Agency, Washington, DC. Office of Water. Report number ADA507297. http://www.epa.gov/ada/gw/pdfs/insituchemicaloxidation_engineering_issue.pdf

- Jacob H-E (1970) Chapter IV Redox potential. *Methods in Microbiology* 2:91-123
- Kasai Y, Kodama Y, Takahata Y, Hoaki T, Watanabe K (2007) Degradative capacities and bioaugmentation potential of an anaerobic benzene-degrading bacterium strain DN11. *Environ Sci Technol* 41(17):6222-6227
- Kurt Z, Spain JC (2013) Biodegradation of chlorobenzene, 1,2-dichlorobenzene, and 1,4-dichlorobenzene in the vadose zone. *Environ Sci Technol* 47(13):6846–6854
- Lee MD, Odom JM, Buchanan RJ Jr. (1998) New perspectives on microbial dehalogenation of chlorinated solvents: insights from the field. *Annu Rev Microbiol* 52 423-452
- Lendvay JM, Loeffler FE, Dollhopf M, Aiello MR, Daniels G, Fathepure BZ, Geghard M, Heine R, Helton R, Shi J, Krajmalnik-Brown R, Major CL, Barcelona MJ, Petrovskis E, Hickey R, Tiedje JM, Adriaens P (2003) Bioreactive barriers: a comparison of bioaugmentation and biostimulation for chlorinated solvent remediation. *Environ Sci Technol* 37(7):1422-1431
- Liang X, Devine CE, Nelson J, Sherwood Lollar B, Zinder S, Edwards EA (2013) Anaerobic conversion of chlorobenzene and benzene to CH₄ and CO₂ in bioaugmented microcosms. *Environ Sci Technol* 47(5):2378-2385
- Lovley DR, Woodward JC, Chapelle FH (1996) Rapid anaerobic benzene oxidation with a variety of chelated Fe(III) forms. *Appl Environ Microbiol* 62(1):288-291
- Mancini SA, Ulrich AC, Lacrampe-Couloume G, Sleep B, Edwards EA, Lollar BS (2003) Carbon and hydrogen isotopic fractionation during anaerobic biodegradation of benzene. *Appl Environ Microbiol* 69(1):191-198
- Mattes TE, Alexander AK, Coleman NV (2010) Aerobic biodegradation of the chloroethenes: pathways, enzymes, ecology, and evolution. *FEMS Microbiol Reviews* 34(4):445-475
- Middeldorp P, De Wolf J, Zehnder A, Schraa G (1997) Enrichment and properties of a 1,2,4-trichlorobenzene-dechlorinating methanogenic microbial consortium. *Appl Environ Microbiol* 63(4):1225-1229
- Ritalahti KM, Amos BK, Sung Y, Wu Q, Koenigsberg SS, Loeffler FE (2006) Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. *Appl Environ Microbiol* 72(4):2765-2774
- Siegrist RL, Lowe KS, Mudoch LD, Slack WW, Houk TC (1998) X-231A Demonstration of In Situ Remediation of DNAPL Compounds in Low

- Permeability Media by Soil Fracturing with Thermally Enhanced Mass Recovery or Reactive Barrier Destruction. ORNL TM-13534 (US Department of Energy) <https://clu-in.org/download/techfocus/fracturing/X231A-3445604441067.pdf>
- Ritalahti KM, Löffler FE (2010) Quantitative Enumeration of *Dehalococcoides* with Real-Time PCR. University of Tennessee <https://www.atcc.org/~media/51773A6D38334DD5A01DEF2B47D12EA3.ashx>
- Tiehm A, Schmidt KR, Pfeifer B, Heidinger M, Ertl S (2008) Growth kinetics and stable carbon isotope fractionation during aerobic degradation of *cis*-1,2-dichloroethene and vinyl chloride. *Water Res* 42(10-11):2431-2438
- USGS (1990) Groundwater Atlas of the United States: Alabama, Florida, Georgia, South Carolina. USGS HA 730-G. http://pubs.usgs.gov/ha/ha730/ch_g/G-text8.html
- van der Meer JR, van Neerven AR, de Vries EJ, de Vos WM, Zehnder AJB (1991) Cloning and characterization of plasmid-encoded genes for the degradation of 1,2-dichloro-, 1,4-dichloro-, and 1,2,4-trichlorobenzene of *Pseudomonas* sp. strain P51. *J Bacteriol* 173(1):6-15
- Verge MF, Ulrich RL, Freedman DL (2000) Characterization of an isolate that uses vinyl chloride as a growth substrate under aerobic conditions. *Appl Environ Microbiol* 66(8):3535-3542
- Yan EY, Schwartz FW (2000) Kinetics and mechanisms for TCE oxidation by permanganate. *Environ Sci Technol* 34(12):2535-2541